GENOTYPIC CONTROL OF FEATHER COLOR PATTERN AS DEMONSTRATED BY THE EFFECTS OF A SEX-LINKED GENE UPON THE MELANOPHORES*

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IT HAS been shown by grafting potential melanophores from one embryo to another of a genetically different breed of fowl or species of bird that the color or color pattern produced in the host feather is invariably like that of the donor breed or species (RAWLES 1939; WILLIER and RAWLES 1940). In other words, the coloration or color pattern produced seems to depend upon the genotypic constitution of the donor melanophore. To establish genotypic constitution as the controlling factor it was decided to test the effects of melanophores derived from embryos of a hybrid cross which shows sex-linked differences in the coloration of the plumage. The F_1 hybrids obtained by mating a Barred Plymouth Rock female with a Rhode Island Red male seemed to be ideal for this purpose.[1] In birds from this cross the juvenile and adult contour feathers of the female are non-barred and predominantely either red or black, the relative proportions of which in a given bird and feather are quite variable, whereas in the males they are typically barred black and white like the female parent. The down plumage is black on the dorsal surface of the body in both sexes except for a white feathered area on top of the head in the males.

The purpose of the present paper is (1) to analyze the differences in phenotypic color effects produced in host feathers by grafting melanoblasts from male and female hybrid embryos, (2) to determine whether the sex of the host has any influence on the color pattern produced and (3) to compare the patterns produced in the host chick with patterns developed in the donor chicks which furnished the melanophores. In this way it is possible to determine the extent to which the melanophore acts independently and how far it comes under the influence of the individual feather germs of the host.

METHOD

The method used for introducing the melanophores of the F_1 hybrid embryo into the mesoderm of the right wing bud of the host embryo has been described in a previous publication (WILLIER and RAWLES 1940, p. 178). The White Leghorn embryo served as host in all cases.

The source of the donor melanophores was either head skin (66–86-hour embryos), wing (108-hour embryo) or leg bud skin (132-hour embryo) or mesoderm taken from the head (100-hour embryo) and from the wing bud (95–120-

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hour embryo). The skin ectoderm and mesoderm from the donor embryos at the stages used contain melanoblasts derived from the neural crest. The melanoblasts migrate from the implant and furnish the melanophores for the host feather germs. The graft as such disintegrates in the mesoderm of the host wing bud, leaving as a rule no coherent traces (for detailed evidence see WILLIER and RAWLES 1940, pp. 185–191). One donor usually furnished the melanophores for one, occasionally for two hosts.

After the implant was removed the donor embryos were incubated until their sex could be determined by morphological differences in the gonads and gonoducts or by down plumage differences, the male only having a white feathered area on top of the head. In this way the sex was ascertained for those embryos which did not hatch and for those chicks which died at or shortly after hatching. In case the donor chick hatched and lived, its sex was determined by sex-linked differences in the down and juvenile contour plumage as well as by secondary sex characters.

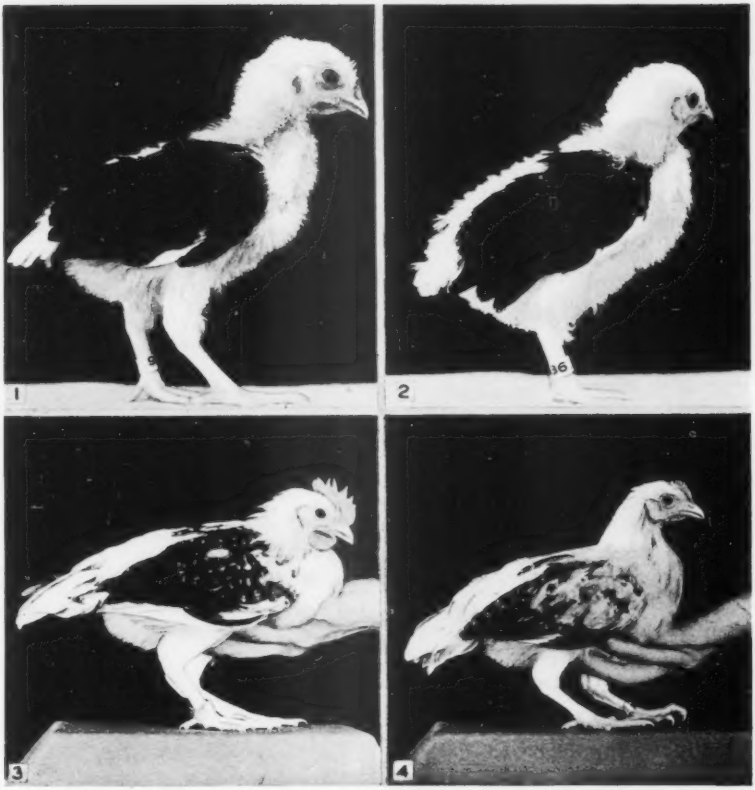
GENERAL STATEMENT OF RESULTS

At all ages (66–86-hour embryos) tested the donor head skin ectoderm when grafted into the wing bud of hosts (66–83 hours) can produce donor-colored (black) pigment in the down feathers of the host wing. Of a total of 107 host chicks examined 83 showed positive and 24 negative results. Thirty-eight of the hosts which hatched were positive and nine were negative. The sex of the donor embryo furnishing the melanophores was ascertained for 16 of the hosts which hatched. Of these four were male on female hosts, three male on male hosts, five female on female hosts and four female on male hosts. Positive results were obtained in all but two cases. Three host chicks died before the juvenile contour plumage had completely emerged. Additional cases in which the sex of the donor was not ascertained are included in table I of a former paper (WILLIER and RAWLES 1940, p. 180).

Pieces of skin ectoderm from the wing bud of 108-hour donors implanted into host embryos of the same age gave a donor coloration of the down plumage in five out of seven embryos. The one embryo which hatched showed negative effects. Positive effects were also produced by grafting a piece of skin ectoderm from the leg bud of donors incubated 132 hours into hosts of the same age. Of a total of six hosts which received grafts two, examined just before hatching, showed the black coloration of the donor and four died before the down plumage emerged.

Head mesoderm grafted from a 100-hour donor embryo into 71-hour hosts produced a donor-colored feather area in three out of five cases. One of these hatched and developed juvenile contour plumage of female coloration.

Wing bud mesoderm from embryos ranging in age from 95 to 120 hours introduced into hosts of 65 to 83 hours incubation likewise has the capacity to produce a black-down area. Of a total of 143 operated upon, 55 died before feathering, 44 showed positive and 44 negative results. Eighteen of the hosts which hatched were positive and 22 were negative. In eight of the positive



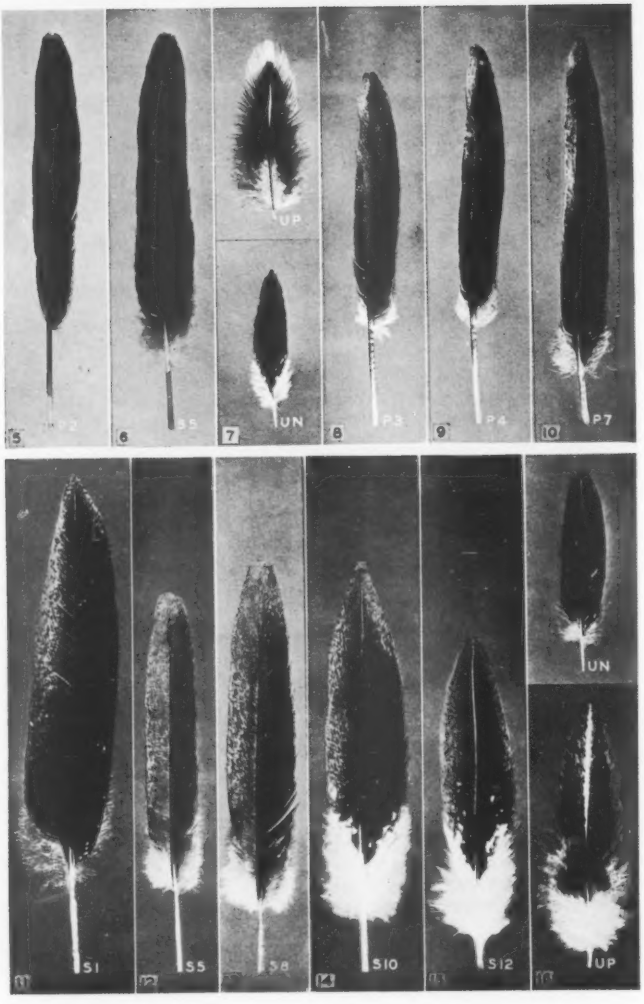
EXPLANATION OF PLATE I

FIGURE 1.—A 16-day old White Leghorn male chick showing non-barred coloration of the female donor. Produced by grafting melanoblasts from a F_1 female embryo of 83 hours into the base of the wing bud of the host embryo at 76 hours.

FIGURE 2.—A 17-day old White Leghorn female chick showing non-barred coloration of the female donor. Produced by grafting melanoblasts from a F_1 female embryo of 84 hours to the wing-bud base of the host embryo at 77 hours.

FIGURE 3.—A 43-day old White Leghorn male chick showing barred plumage, produced by grafting melanoblasts (in wing bud mesoderm) from a F_1 male embryo of 107 hours into the wing-bud base of the host embryo at 72 hours. Selected individual feathers from this host are illustrated in figures 23, 25, 27 and 29 (uppermost feather).

FIGURE 4.—A 44-day old White Leghorn female chick showing barred plumage, produced exactly as described for the chick shown in figure 3. See figures 17–22, Plate III for selected individual feathers from this host.



EXPLANATION OF PLATE II

In Plates II and III the primary and secondary juvenile flight feathers are designated respectively as P and S. The numeral which follows indicates the position of the feather with reference to the axillary or wrist feather—that is, from this feather the primaries are numbered distally and the secondaries proximally on the wing. Upper and under wing coverts are designated respectively as up and un.

The outer and inner vane halves are respectively left and right in all feathers illustrated except in those of figures 24, 26 and 28 where they are reversed (that is, left wing feathers).

FIGURES 5–7.—Juvenile wing feathers of the black type of female donor coloration, all from the same male host. Figure 7 un is a donor-host color mosaic.

FIGURES 8–16.—A series of juvenile wing feathers of the black-red type of female donor coloration, illustrating variation in pattern produced by different feather germs of the same female host. Note that S10, S12, up and un are donor-host color mosaics, the donor coloration having been replaced by the white of the host in the proximal portion of the vane.

cases, the donor although failing to hatch was old enough for its sex to be ascertained. In ten cases both donor and host hatched. Six of these unfortunately showed no donor-colored areas. However, two donor-host pairs in which the host showed donor coloration lived until after the juvenile contour plumage emerged.

After hatching the black-colored down feathers of the wing and breast of the host chick are gradually replaced by pigmented contour feathers of the juvenile plumage. These exhibit two distinct classes of color patterns, one which is non-barred like the female and the other barred like the male donor control chicks (*cf.* figs. 1 and 2 with 3 and 4 of Plate I).

THE FEMALE NON-BARRED PATTERN

Experimental

Contour feathers with non-barred coloration have been produced in sixteen hosts. In nine of these the sex of the donor chick was ascertained to be female. It is inferred, therefore, that in each of the remaining hosts having non-barred feathers, the melanophores came from a female donor embryo.

With respect to coloration of the contour feathers two types of hosts are distinguished, those in which the feathers are predominantly black and those in which the feathers usually exhibit a mixture of red and black pigments. In the black type the primary and secondary flight feathers as well as the upper and under wing coverts are usually uniformly black without red pigmentation, but upper coverts occasionally occur with red portions in the shaft (see figs. 5, 6 and 7, Plate II).

In the black-red type the feathers although mostly black show variable quantities of red pigment in the different host feathers (see figs. 8–16, Plate II). Typically in the flight feathers the outer vane half (portion on side of shaft away from body axis) is a mixture of black and red pigment—that is, a red background in which are scattered small flecks of black pigment, producing a stippled pattern (see figs. 11–12). The outermost edge may be a pure red, entirely free of black pigment. In general the black pigment flecks occur closer and closer to one another in the direction of the shaft, grading into a solid black zone next to it. In some feathers the red-black pattern may extend up to the black shaft. The inner vane half (portion on side of shaft toward body axis), on the other hand, is predominately black except in the apical region where the pigment pattern resembles closely that of the outer half of the vane. The outermost edge of the inner vane, especially in the secondary flight feathers, is fringed with red pigment.

In the upper coverts the combination of black and red is somewhat different from that found in the flight feathers. The apical half of the feather vane has a red and black pigment pattern similar to that found in the flight feathers whereas the basal half is predominately a gray-black. The red pigment in the apical half tends to be localized in the shaft and in a narrow zone on each side of it. The red color may extend for a short distance in the shaft of the basal half (see fig. 16 up).

The under coverts exhibit a similar condition except that there is much less red pigment. Such feathers thus appear predominately black, the red being chiefly localized in the apical third of the shaft (see fig. 16 un). In some under coverts there is still less red pigment which is confined to the outer margins of the apical portion of the feather vane.

It is thus seen that there are two classes of female donors, the melanophores of each producing its own type of color pattern. Feathers having the black-red stippled patterns are much more commonly produced than those colored uniformly black. The sex of the host apparently has no effect since each type of pattern has been formed on hosts of either sex (*cf.* with results obtained by DANFORTH (1929) showing that the non-barred coloration in female skin grafts is not affected by the sex of the host).

F_1 female control

An examination of the juvenile contour feathers of seven F_1 control females shows that the individual birds differ with respect to the relative amount of black and red pigment. Three birds have predominantly black plumage. The axillary, primaries, secondaries, upper and under wing coverts are usually uniformly black. Occasionally a small amount of red pigment occurs in the wing coverts where it is usually confined to the apical half of the shaft. In only one individual was any red pigment found in the flight feathers. In this bird the secondaries have small amounts in the apical third of the vane, especially in the outer half. In those chicks (four examined) having a greater quantity of red pigment in the plumage, the amount varies depending upon the position of the feather. Usually the primary flight feathers are blacker than the secondaries. The red pigment is intermingled with the black and is typically confined to the outer vane half and to both halves of the apical ends of the primaries. In the secondaries the outer half of the vane and both outer and inner halves of the vane of the apical portion show an irregular mixture of red and black pigment forming a stippled pattern. The red pigment spreads proximally in the later emerging secondaries, so that in S12 and S13 (for system adopted in numbering the flight feather see Pl. II; see also WARREN and GORDON 1935) both inner and outer vanes are stippled throughout their entire length. Both the upper and under coverts have a mixture of red and black pigmentation in the upper half of the feather. The shaft and bases of the barbs may be almost exclusively red. The basal half of the covert is predominantly black. In the axillary and secondaries (also in P9 and P10) of one individual the black pigment, especially in the outer vane, tends to be regularly distributed in the form of a penciled pattern consisting of one or two longitudinal black stripes on a red background. The penciled pattern is more distinct and more likely to be double striped in late emerging flight feathers than in those emerging relatively earlier. Within the axillary-secondary series a perfectly graded series of intermediate patterns is found between stippled (S2, the first to emerge) and penciled (S12, S1, and A, among the last to emerge), paralleling closely the time order of development.

Thus, generally speaking, there are three classes of control females with respect to pigmentation of the wing feathers. By their distinguishing characteristics the feathers of such females may be referred to as (1) uniform black, (2) stippled and (3) penciled. The black and stippled types correspond closely to the two classes of donor-colored feathers produced in the host chick and like them show similar variations in color pattern depending upon the position of the feather on the wing. The penciled pattern has not been produced in any host feather.

THE MALE BARRED PATTERN

Experimental

Fifteen hosts with alternating black and white bars in the contour feathers have been examined. In seven of these the sex of the donor embryo or chick was proved to be male. In the remaining hosts having the same color patterns it is deduced that the melanophores were furnished by male donors also.

With respect to the color pattern produced, two types of hosts are found, those in which all the donor-colored feathers show a barred pattern not unlike the typical barred pattern of the female parent of the hybrid donor, and those in which the typical bars are limited to certain feathers only.

In those hosts having the typical barred patterns in the juvenile contour plumage, the specific pattern which is formed varies with the position of the feather on the wing and breast and also from host to host (see figs. 17–22, 23, 25, 27 and 29 of Plate III). In general the primary flight feathers (P_1, P_2 and P_3), which are the first to emerge from the follicle, are almost wholly black, only the shaft showing more or less distinct alternating bands of white. This condition is illustrated in P_2 (fig. 23) which also has a narrow white bar at the apex. In the primaries which emerge successively later and later there is usually a progressive increase in the number of alternating white and black bands in the basal portion of the vane. Beginning with primary four where only one white bar is present in the vane, the number increases in the successive feathers until P_7 where the barred pattern extends over the basal half of the feather. In some hosts the vane barring begins with P_7 and becomes a little more distinctive in the next emerging primary, P8 (fig. 17). A similar increase in the number of bars is found in the secondaries in accordance with the time of their emergence. The first three or four secondaries are nearly uniformly black except for a single faint white bar in the apical portion of the vane and for several white bars on the shaft (figs. 18, 19 and 25). Beginning with either S_4 or S_5, depending upon the host, a white bar makes its appearance in the basal-most part of the vane. In successive feathers—that is, S_5 or S6, S7, S8, etc., the number of bars increases and spreads apically until in S10, S11 or S12 (varying with the host) the vane is more or less barred throughout its entire length (fig. 20, 21 and 27). In the secondaries beyond S11 the pattern is more distinct—that is, the bars are more sharply defined and straighter. The faint white bar noted in the apex in the first three secondaries becomes gradually more distinct in the later emerging ones. Ultimately in S10 it usually forms a

part of the barred pattern which has gradually spread apically from the proximal part of the vane (figs. 20 and 21).

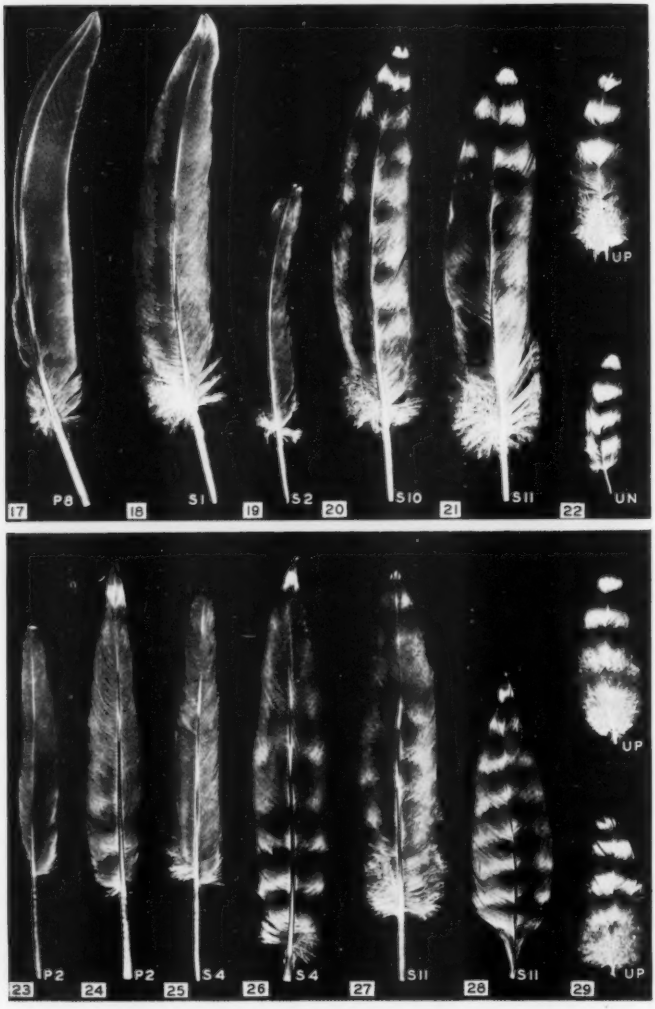
In the breast feather, in contrast to the late emerging secondaries, the amount of black pigment is much less. The dark band is light gray instead of gray-black and the light band pure white instead of gray-white. Furthermore, the light bar is wider than the dark, each being wider than the bars of the secondaries. In the wing coverts there is still a different type of barring (see figs. 22 and 29 up). Here the white and black bands are distinct, nearly of equal width and are much narrower than in the breast feathers or the late-emerging secondaries.

Three hosts have been examined in which the barred pattern is distinct but limited in its occurrence. The typical barred pattern is limited to the upper and under wing coverts and is like that just described above for corresponding coverts. The flight feathers have mostly a non-barred pattern. In the first two or three primaries the feather is of a uniform black coloration, no barring showing in either the shaft or vane. In primaries four to seven the wide inner vane is of uniform black color whereas the narrower outer vane exhibits an irregular pattern of white, black and tan colored areas. This irregular or stippled pattern increases in extent with the order of emergence of the feathers, being greatest in the last one to appear. In P9 a black and white barred pattern is exhibited in the proximal fourth of the vane. One or two white bars somewhat far apart may appear in the shaft of all the primaries except the first two.

The secondary flight feathers exhibit a pattern which varies with the time of their emergence. As a rule the inner vane is a uniform black color whereas the outer vane is mostly black except for its outer half which is stippled with white and tan. In the first secondary to emerge—that is, S2, the stippled zone is confined to the second fourth of the apical half of the vane. In the successively emerging feathers it spreads gradually apically and proximally until in S8 it extends throughout the entire length of the feather. Also following the same order the amount of black pigment gradually diminishes until in S10 or S11 the zone is predominantly of a tannish white hue flecked here and there with black pigment.

Beginning with either S9 or S10 the apical sixth of the feather shows a few grayish white bars in the black background. Typically there is just one distinct white bar in the shaft of all the secondaries. In S2 it is located in the upper fourth of the proximal half of the feather. In the successively emerging secondaries its locus gradually shifts apically until in S10 it is near the apical end. Apparently this white bar has risen simultaneously in all of the secondaries concerned.

Summarizing, it is seen from the kinds of patterns produced that the donor male embryos fall into two classes, the melanophores from one producing a typical barred pattern whereas those from the other produce a barred pattern in only certain feathers of the host. In the latter type the outer vane is stippled with black, tan and white areas. The former type is the more common. Each pattern type has developed on hosts of either sex. (See DANFORTH (1929) for



EXPLANATION OF PLATE III

FIGURES 17–22.—Juvenile wing feathers showing variation in barred pattern produced by different feather germs of the same host (from female host chick shown in figure 4).

FIGURES 23–29.—Juvenile wing feathers arranged in homologous pairs to show differences in quality of barred pattern formed in host and in donor which furnished the melanophores. Of the pairs of flight feathers illustrated the one to the left is host and the one to the right is donor. Of the wing coverts shown in figure 29 the lowermost is donor and the uppermost is host. The host feathers are from the chick shown in figure 3, Plate I.

evidence showing that the sex-linked barred pattern in male skin grafts is manifested independently of the sex of the host.)

F_1 male control

The barred pattern in the juvenile contour feathers of the thirteen control F_1 males examined shows considerable variation. It varies from bird to bird and also depends upon the position of the feather on the wing and breast. In some birds the five or six primaries which first emerge are almost wholly black, only the shaft showing alternating bars of white; in others these feathers have also a white band running across the apex of the vane and indistinct barring in the proximal portion of the vane (fig. 24). In the later emerging primaries, such as P7, P8, P9 and P10, marked differences in pattern on the two sides of the vane are seen. In the outer vane which is narrower than the inner vane the alternating bands of white and black are very distinct, whereas in the wider inner vane they may be either a uniform black or gray black or a gray black in which still lighter colored white bars may be discerned, particularly in the proximal half of the vane. In some individuals the barred pattern occurs in the proximal half of the inner vane as well as throughout the length of the outer vane. Usually these bars are less distinct (gray, not white) than those of the outer vane.

In the secondary flight feathers the pattern varies depending upon the time of emergence. In the first to emerge—that is, S2, the apical one-fifth or sixth of the feather is distinctly barred whereas the proximal four-fifths is either somewhat uniformly black or faintly barred. The bars usually extend farther proximally in the shaft than in the vane. In the feathers which emerge successively later the barred pattern gradually extends proximally, beginning in either S8 or S9 to be barred throughout. All of the remaining secondaries—that is, S10 to S14, are likewise completely barred. In these, the bars become successively more distinct and the light bars become progressively whiter. The apical-proximal order of extension of barring just described may be reversed. In one donor chick of the two donor-host pairs which lived, the proximal barring becomes distinct early in the secondary series and in the successive feathers spreads apically while the apical bars remain more or less unchanged. An intermediate condition in this extension is shown in S4 of figure 26. Beginning with S10 the apical and proximal patterns become continuous so that this feather and the succeeding ones (S11, S12 and S13) are barred throughout (see fig. 28). In S1, the last to emerge, the pattern is very variable ranging from a uniform black except for white bars in the shaft, indistinct gray bars on the outer vane, to gray bars on both sides throughout the length of the vane. The axillary feather typically shows a barred pattern at the proximal and apical ends of the vane while the intervening portion of the vane and shaft may be faintly barred or not barred at all.

In some birds there are certain feathers, such as the axillary and particularly the first two or three secondaries, which exhibit an irregular mixture of black and white or tan areas in the outer vane. This irregular pattern may be confined to the margin of the vane only or it may extend up to the shaft. The por-

tion between it and the shaft is usually a uniform black, although it may occasionally be barred. The pattern in the inner vane of such feathers is like that described above for homologous feathers. In such cases the wing coverts as a rule have distinct black and white bands, the latter being wider than the former, particularly in the apical half of the feather. In some individuals, particularly those showing the irregular mixture of black, tan or white areas in the axillary and secondaries, the covert and breast feathers occasionally show mosaic color patterns. The apical portion may be barred black and white, or a uniform white, the middle portion red, and the proximal fluffy portion of non-barred black.

Summarizing, it is seen that the flight feathers of the wing show much variation in the barred pattern formed. It not only varies from individual to individual but varies in any individual depending upon the position of the feather. In certain birds the axillary and secondaries exhibit in the outer vane an irregular mixture of black and tan or white. In such birds the wing coverts and breast feathers show a greater tendency to form aberrant color patterns.

Thus it is seen that the barred patterns of wing feathers of the control hybrid males show variations similar to those produced in host wing feathers by grafting melanophores. Like some of the control birds, some hosts have feathers which show an irregular mixture of black and tannish white colors. No distinctly red areas appear in the coverts of the host birds, however.

PHENOTYPIC EXPRESSION DEPENDS PRIMARILY UPON THE GENOTYPIC CONSTITUTION OF THE MELANOPHORE

The results show clearly that the color pattern produced in the host feather depends upon the genotypic constitution of the donor F_1 hybrid embryo furnishing the melanophores. Genetically the melanophore producing the female non-barred pattern has the constitution *b s*, *b* and *s* representing, respectively, the non-barring and non-silver alleles of two dominant sex-linked genes, barring (*B*) and silver (*S*); in addition it is heterozygous for the dominant autosomal gene E^m which governs the extension of black pigment (for the genetic constitution of the Barred Rock and R. I. Red parents see AGAR 1924; DUNN 1922, 1923, 1924 a, b; HALDANE 1921; HAYS 1926; and WARREN and GORDON 1933), hence we should expect it to produce a non-barred uniformly black host feather Invariably a non-barred pigmentation has been produced in the host feather. With respect to pigmentation effects, however, the melanophores of the individual donors fall roughly into two main classes (a) those which produce a uniformly black coloration in all (with rare exceptions) host feathers and (b) those which produce a black and red pattern in all host feathers (see Plate II). In those hosts having uniformly black feathers, apparently E^m has expressed itself as a nearly complete dominant in the melanophores. On the other hand, the dominance of E^m appears to be less completely expressed in the melanophores (probably as a result of other factors affecting the dominance of E^m) producing a black and red pattern in host feathers.

The melanophore of the male donor possesses the genes for barring (*B*) and

silver (*S*) on one of the X chromosomes and the genes for non-barring (*b*) and non-silver (*s*) on the other (*BS bs*) as well as the autosomal genes $E^m e^m$. Since *B* is dominant to *b* we should expect the male melanophores to produce a barred pattern in the host feathers. Furthermore, in the presence of E^m and *S* (known to suppress red coloration) the *B* gene should produce black and white stripes. In the majority of cases typical black and white barred patterns in the host feathers have been produced. A careful examination of these feathers shows the complete absence of any red pigmentation. It becomes evident therefore that not only *B* but also *S* and E^m express themselves in the melanophore. In three hosts, however, the melanophores have produced a pattern in which distinct barring is limited to the wing coverts, the flight feathers showing an irregular mixture of black, white and tan colors on the margin of the outer vane half. These differences in pattern produced cannot be attributed to differences in the feather germs since the same homologous feathers are affected in all hosts and these two types of patterns occur in the juvenile plumage of some of the control hybrid male birds. It is inferred, therefore, that there are two classes of males whose melanophores vary with respect to factors affecting the dominance of the three main genes concerned.

The general absence of red pigment in the barred host feather is not an unexpected result in view of the fact that it appears only infrequently in the down and juvenile contour plumage of control males. In some control male chicks the down feathers, particularly on the head, are reddish-black in color. The juvenile contour feathers on the wing and breast are generally free of red pigment. Only rarely does a wing covert or breast feather have a small area of red color (resembles R. I. Red) in the vane. Such feathers are color mosaics consisting of a barred black and white tip, a red middle portion and a non-barred black portion. Barred feathers are also found in which a tan coloration occurs in both the black and white bars. This type occurs somewhat frequently. Similar exceptional types of color mosaics in fowls heterozygous for *B* and *S* have been analyzed by SEREBROVSKY (1926) as evidence for somatic crossing over.

THE SPECIFIC COLOR PATTERN PRODUCED DEPENDS UPON THE INDIVIDUAL FEATHER PAPILLAE

The color pattern produced by the transplanted melanophores, whether genotypically male or female, varies more or less widely in the different feathers of a host. Of the two types of female patterns the black one shows much less variation than the black-red pattern. The black type is usually a uniform grade of black throughout the vane and shaft and in the black parts of feathers which are mosaics of donor and host colors (figs. 5, 6 and 7). This is true of the flight feathers and most coverts. Figure 7 up shows an exceptional covert in which a portion of the shaft and the bases of adjoining barbs are colored red (white in photograph). Although the feathers are generally black to the naked eye an examination with a dissecting microscope reveals the presence in some of them of a few scattered flecks of red pigment. In the secondary flight feathers of some hosts the red flecks tend to be more common in the apical portion

of the vane especially the outer half. The primary flight feathers are less likely to show the red flecks.

The black-red type of coloration exhibits a much wider variation in patterns formed (figs. 8–16). In the primary flight feathers (P1–P5) which emerge first, the vane is black throughout except for its apical one-sixth which exhibits a mixture of red and black pigment. The amount of red pigment is greater and extends proximally farther in the outer than in the inner vane half (figs. 8 and 9). The later emerging feathers (P6–P10) show the same black coloration except that the amount of red pigment gradually increases, spreading toward the shaft and proximally in the successively emerging feathers, until, in the last to emerge, the red in the outer half of the vane greatly exceeds the amount of black and extends practically the entire length of the feather. See fig. 10 for the extent of spreading of the red pigment in P7.

The secondary flight feathers show a gradual increase in the amount of red pigment paralleling the order of their emergence. In S2 which emerges first the red is mostly confined to the apical two-thirds of the outer vane half and to the apical one-tenth of the inner vane half. In the feathers which emerge still later the amount of red increases, extends farther basally in each vane half until in S12 or S13 the degree of red pigmentation is approximately the same on the two sides (see figs. 12, 13, 14 and 15). It is a little more concentrated in the outer vane. The red pigmentation in the outer vane extends almost to the fluffy part of the feather before it spreads basally in the inner vane. As the red pigment spreads in an apico-basal direction in the inner vane it is accompanied by an extension of red pigmentation in the shaft of the feather (*cf.* figs. 14 and 15). In S1 (fig. 11) and S10 (fig. 14) which emerge at approximately the same time the distribution of red pigment is somewhat similar. In some cases the red pigmentation increases in the outer vane to the extent that very little black pigment remains visible. Its increase parallels the order of emergence of the secondaries from two to thirteen, being greatest in the last one (S13).

Some of the coverts or their donor-colored parts are entirely black with the exception of a fringe of red pigment along the margin of the inner vane half. Others are black except for the upper third or half of the shaft which is red (fig. 16 un). In the apical portion of the vane of these, the black is somewhat diluted with red pigment, especially on the inner side. In still other coverts the amount of red pigment is much greater in the upper half of the vane and shaft, the basal half of the feather being black (fig. 16 up). The red pigment in these tends to be more or less symmetrically distributed in the vane and freer of black pigment in the shaft and bases of the barbs on each side. The order of emergence of the coverts has not been studied, so no correlation can be made between this and the quality of pigmentation of the feather.

In effect the same kind of variations in host feathers is found when melanophores from the F_1 hybrid male embryo are grafted (figs. 17–22, 23, 25, 27 and 29). In general the primary flight feathers are darker than the secondaries. The barred pattern formed in each primary and secondary is distinctive, the variation depending upon the time of its emergence as noted above. In general the

flight feathers which emerge first are blacker and the barring less definite than in the later emerging feathers. In the breast feather the white and black bars are broader than in S10 to S14. In the coverts the white and black bars are of nearly equal width and much narrower than in the breast feathers.

Thus we see that though the melanophores all came from the same region (head skin) of the male or the female donor embryo the pattern produced varies with the time of emergence and the position of the feather on the body of the host. It is apparent, therefore, that the individual feather germ determines the quality of pattern produced. Each feather germ produces its own particular pattern. Furthermore, each feather germ of the White Leghorn host interacts with the transplanted melanophores to produce a color pattern similar to that formed by its homologous feather germ in the donor control chick. This shows that homologous feather germs of these two breeds are similar in their physiological organization. That they are similarly organized in most if not all of the common breeds of fowl is probable (for other examples see WILLIER 1941).

THE MECHANISM OF COLOR PATTERN FORMATION

In order to understand the mode of formation of the coloration in the host feather it is first necessary to consider what types of melanophores are found in the two parents and in their F_1 progeny. From an examination of the feather germ of both embryos and hatched chicks of various ages and of *in vitro* cultures it is clear that the Barred Plymouth Rock has only black melanophores whereas the R.I. Red has two types, one black and the other red. In the majority of F_1 females both red and black melanophores are commonly found in the feather germs. In some individuals, however, the melanophores are all black except for a very few red ones. The black ones contain and deposit black rod-like granules while the red ones contain and deposit only small globular granules of a reddish color. In the F_1 male only black melanophores as a rule are found in the majority of the feather germs in young and adult birds. However, in aberrant feathers of the juvenile and adult plumage red pigmentation occurs showing that red melanophores are capable of being formed. In down feathers of the head, particularly those about the beak, red melanophores as well as black ones may be found in some male individuals.

In considering the mode of formation of the non-barred pigmentation in host feathers, it is first of all essential to note that the hybrid females differ among themselves with respect to the kind of melanophore they are capable of producing. Apparently, a female donor has the potency for forming nearly all black melanophores when the dominance of E^m is complete or nearly so. On the other hand, when the dominance of E^m is less complete the female donor has the capacity to form readily both black and red melanophores. Thus differences in the expressivity of E^m in the melanophore appear to be primarily responsible for the variations produced in the character of the pigmentation—that is, whether the feathers of a particular host can produce a nearly complete black coloration or a black-red ("stippled") pattern.

The question next arises as to what extent the genotypic expression of the

melanophores of the hybrid females is dependent upon conditions within the feather germs of the host. In the black type of female coloration little or no evidence is furnished for host effects on the melanophores inasmuch as the feathers are nearly all uniformly black. On the other hand, in the production of the black-red ("stippled") pattern which involves the differentiation of both black and red types of melanophores, the conditions of control within the feather germs are more clearly revealed. An examination of the various cases of this type shows that within the same host the donor-colored feathers exhibit a variety of patterns in the distribution of red and black pigment. For the flight series three generalizations are of significance. (1) The relative amount of black pigment per feather is greater in the primary than in the secondary flight feathers. In some individual hosts all of the primaries are wholly black except for traces of red pigment at the extreme tips; in others this distribution is characteristic of the first two or three primaries only, the remainder having red pigment mixed with black not only in the tips but in the outer vane as well. (2) Red pigment appears almost invariably at the apex of all the flight feathers; and extends proximally to the fluff in the outer vane of the late emerging primaries and of all the secondaries. In the late emerging secondaries (that is, S11 and S12) it extends proximally in both vane halves up to the fluffy portion which is grayish black. (3) Irrespective of whether the inner or outer half vane is the wider, the red pigment is invariably either limited to or greater in amount and extent in the outer vane half. These observations show that the feather germs vary greatly among themselves in their control of the direction of differentiation of the melanophores from a female donor of the black-red type. Each one appears to be specifically different from the other in its control. The pattern of red and black coloration produced varies with the position of the feather in the flight series. In certain flight feathers or regions thereof the conditions favor the formation of black melanophores almost exclusively and in others of many red melanophores and relatively few black ones.

Can such a distribution in pigmentation be correlated with differences in growth rate of the different flight feathers? In an attempt to answer this question, the rate of elongation of each juvenile flight feather of control White Leghorn chicks was measured at two day intervals from the beginning of emergence (tip of feather at mouth of follicle) until its completion. The results show that the primaries as a whole increase in length a little more rapidly than the secondaries. This difference is particularly well brought out by comparing the early emerging primaries with the late emerging secondaries. For example, P1 has a rate of 3.14 mm per day while S11 has a rate of 2.04 mm per day. Comparing these two feathers from the same host in the experimental series, it is found that the primary is almost entirely black whereas the secondary has large quantities of red pigment in both outer and inner vanes. Thus a rapidly growing feather seems to present more favorable conditions for the deposition of black pigment than a slower growing one. The formation of the red tip, common to most of the flight feathers, may be correlated with a slow rate of growth. LILLIE and JUHN (1932) have shown for regenerating breast and saddle

feathers of the Brown Leghorn fowl that the rate of elongation is at first quite slow and subsequently quite rapid—that is, the apical end is growing more slowly than more proximal portions. Similarly, in the White Leghorn fowl the rate of elongation of flight feathers measured after emergence begins is relatively slow at first, reaching a rapid rate shortly after.

That rate of feather elongation is not the only factor operating is shown by the fact that although two flight feathers in different loci may have nearly the same rate of elongation, the color patterns formed are very different. Two examples will suffice to illustrate this point. In one female host primary five is all black except for its apical end and margin of outer vane which show small amounts of red pigment. Secondary five contains in the outer vane considerable quantities of red pigment extending from apex to almost the fluffy portion while the inner vane is black throughout except for a red tip. In the control female chick both of these feathers grew at the rate of 2.50 mm per day. In one male host P_3 is entirely black except for a small amount of red pigment in the outer vane of the apex, while S_4 contains red pigment on both sides of the shaft at the apex, on the outer vane from apex to nearly the level of the fluff, and black pigment in the outer vane except at the apex. In the control male chick P_3 and S_4 grew respectively at the rate of 2.18 mm and 2.17 mm per day.

If black pigmentation is to be correlated with a rapid growth and red pigmentation with a relatively slower rate, we should expect on the basis of the hypothesis of MONTALENTI (1934) that red pigment would differentiate in asymmetrical feathers in the short side of the vane where the rate of barb formation is slower and black pigment in the long side of the vane where the rate of barb formation is higher. This is not the case, since in a primary flight such as P8 red pigment occurs on the short side of the vane and black pigment in the long side of the vane half, whereas in a secondary such as S1 the reverse is the case. Thus red pigmentation actually appears in the outer vane half irrespective of whether it is wider or narrower than its opposite vane side.

It becomes evident, therefore, that some physiological conditions other than growth rate are responsible for the differences in color pattern found in the flight feathers. These feathers, located in different positions in the flight series, exhibit constant and often striking differences in their shape (length and width of feather, curvature of shaft and asymmetry of vane halves), suggesting that each feather germ has a morphogenetic property peculiar to it. Since JUHN, FAULKNER and GUSTAVSON (1931) have shown for the Brown Leghorn capon that the threshold of response of a regenerating feather germ to female sex hormone varies from tract to tract and from feather to feather within a tract, it seems probable that the epidermal collar of each feather germ in the flight series has a specific reaction threshold. The threshold may be relatively high in one and relatively low in another. Furthermore, the right and left vane halves of a feather germ may differ in reaction threshold, one half being either higher or lower than the opposite half depending upon the position of the feather.

That regenerating secondary flight feathers of the male or capon Brown

Leghorn exhibit among themselves different orders of the reaction gradient (for pigmentation) to increasing concentrations of female hormones is shown by the work of FRAPS (1938). According to him the order in secondaries may be ventrodorsal, which is the reverse of the dorsoventral order characteristic of breast and saddle feather germs, or it may be more complex—that is, proceeding ventrally and dorsally from a mid region in lateral vane halves. It has been argued elsewhere (WILLIER 1942) that the response of the melanophores is governed by a gradient in reaction threshold and reaction time along the barb ridges, hence any variation in the order or direction of the reaction gradient within the feather germ is to be correlated with any variation in color pattern produced. The order of reaction gradient in a given feather germ plays a decisive rôle in determining the specific color pattern formed. Before we can proceed much further with the analysis of color patterns in flight feathers, a study of the variation in reaction gradients among the various flight feathers should be made.

The individual secondary flight feathers often exhibit in the vane halves, more commonly in the outer half, a gradient in the distribution of relative amounts of red and black pigment (see fig. 11). The red pigment tends to predominate in the apical ends of the barbs, gradually decreasing in amount toward the rachis. As the red pigment thus diminishes in amount the black pigment increases and appears in the form of small black flecks which become larger and closer together, culminating in a solid black. This gradient in red-black pigmentation may extend all the way to the rachis, which is black, or stop short of it, whereupon the basal portions of the barbs and rachis are wholly black. If this gradient is examined in the light of the principle of the gradient in threshold and in reaction time along the barb ridges of a developing feather, established by LILLIE and his co-workers, we have a possible mechanism for controlling the direction of the differentiation of the melanoblasts. In the apical tip of the barb where the threshold of response is high and the reaction time short, red pigment only appears in the intermediate zone of the barb where the threshold is lower and the reaction time longer, both red and black melanophores develop, and in the axial portion of the barb where the threshold is lowest and the reaction time longest, only black melanophores differentiate.

Let us examine next the course of differentiation of a precursor melanophore of a hybrid female which has the potency for forming both red and black melanophores. At the time of entrance into the epidermis of the feather germ it may be assumed that such precursor melanophores are either (1) already differentiated into two potential cell types or (2) not fully differentiated—that is, the precursor is a single cell type possessing the capacity (a) to form either a red or a black melanophore or (b) to pass through an intermediate red stage in the formation of a black melanophore. If two potential cell types exist prior to their entrance into the epidermis it would be necessary to assume that local differences within the epidermal substratum determine the number of each kind formed. There is little or no evidence favoring this hypothesis. According to the investigations of FOULKS (1942, 1943) on the Barred Plymouth Rock,

the precursor melanophores are already in the basal part of the epidermal collar of a regenerating papilla before they form pigment granules. Cells with pigment granules do appear in the dermal papilla and in the boundary between epidermis and dermis but these have apparently differented *in situ*. That fully differentiated melanophores, however, do multiply in the epidermis of a down feather germ, where melanin deposition is in progress, has been shown by WATTERSON (1942).

It seems much more probable that at the time of invasion the precursor melanophores of the black-red type of female donor are not fully differentiated cells but cells whose direction or stage of differentiation attained are dependent upon local influences within the epidermal substratum. The hypothesis that the precursor melanophore is a single cell type which has the capacity to synthesize either red or black melanin granules depending upon external influences is attractive from a chemical point of view. RAPER (1932) has suggested that several intermediate chemical substances are formed in the oxidative action of tyrosinase on tyrosine. One of these, formed early in the reaction, is a red colored substance (a quinone of an indole derivative). Upon further oxidation this becomes colorless and finally black melanin precipitates out as the end product. From this it has been inferred that the red melanophore is one in which the red granules represent an intermediate product in the synthesis of black melanin. Some influence from the epidermal substratum blocks the differentiation of the precursor melanophore at the red stage. Under different conditions in the substratum the synthesis might continue to the definitive stage and a melanophore containing black melanin granules would differentiate.

This view, although theoretically interesting, has little evidence in favor of it. In the Rhode Island Red and New Hampshire Red breeds of fowl, according to HAMILTON (1941) the regenerating feather and embryonic skin explants show two distinct cell types but no intermediate cell types. No melanophore has been found containing simultaneously both black and red types of melanin granules. No granules of an intermediate nature between these types have as yet been found. Furthermore, the red granules are globular and the black ones are rod-shaped, entirely different in their form. It hardly seems probable that the red globular granule could transform into a black rod-shaped granule. On this point LLOYD-JONES (1915) who made a study of the development of pigment granules in pigeon feathers, states " . . . pigment granules arise directly in the form which they will permanently hold . . . " (p. 476).

The most acceptable view to adopt, therefore, is that the precursor melanophore of the black-red type of female donor is a single stem cell which has the capacity to differentiate into either a red or a black melanophore. This dual capacity appears to be provided when the dominance of E^m is sufficiently weakened by the rest of the genotype, and differs only in degree from a more or less unipotent condition (black melanophores) provided when the dominance of E^m is complete or nearly so. The unstable genotypic constitution may be thought of as providing the precursor melanophore with alternative potencies or pathways along which the differentiation processes may proceed. However,

which potency is actually realized depends upon regional physiological differences in the epidermal substratum of the feather germ. In other words, a precursor melanophore in which the dominance of E^m is sufficiently weakened by modifying factors will become fixed as a red or a black cell type by specific influences emanating from the epidermal substratum. If fixed as a red type it synthesizes red globular granules, if fixed as a black type it synthesizes black rod-shaped granules. Apparently once a precursor melanophore is fixed as a red type it has no further capacity to develop into a black type. That the type of melanophore segregated is distinctive and remains so has been shown by DANFORTH (1937). In papillae of graft mosaic feathers, obtained by transplanting skin from Buff Leghorn to Jersey Black Giant in newly hatched chicks, he found two distinct types of melanophores, one characteristic of the donor and the other of the host chick. The donor melanophore contains small, nearly spherical granules, while the host one contains black, rod-shaped granules. With respect to their distinctive behavior, he states: "In the anlagen of mosaic feathers each chromatophore is clearly and unequivocably of one or the other type in respect to its pigment granules and no intermediate, or different, forms have been observed, even when two contrasted cells were in close proximity or in actual contact. Nevertheless, the two kinds of granules are often found mixed together in single epidermal cells of future barbs and barbules, indicating that two different chromatophores may feed their products in to the same recipient cell" (p. 463). Furthermore, by crossing Black Minorcas (same kind of black as Jersey Giants) with Buff Leghorns he obtained three types of melanophores, each individual one producing "but one type of granule" differing in shape and color (p. 464).

In a recent paper HUMM (1942) maintains that red pigment cells, after reaching "full differentiation" in long time *in vitro* cultures can, when transplanted to a White Leghorn host embryo, multiply, migrate and produce either red or black pigment in feathers depending upon local skin differences (red in flank, black as well as red in tail feathers). That is, a fully differentiated red pigment cell can under suitable conditions form a black pigment cell. This interpretation is difficult to accept in view of evidence cited above that normally precursor pigment cells enter the developing feather germ before they have pigment granules. It would appear probable, therefore, that some of the red pigment cells either did revert to an undifferentiated state (without pigment granules) or some of the neural crest cells remained in an undifferentiated state in the culture. A histological examination of the "pure culture" of red pigment cells should be made to ascertain whether or not *all* cells are typical pigment cells with red globular granules.

We shall next turn our attention to an analysis of the mode of formation of the barred pigmentation pattern in White Leghorn host feathers brought about by melanophores from the hybrid male donor. The typical barred pattern produced is invariably in accord with the genotypic constitution of the donor male chick which, as was pointed out above, is heterozygous for the sex-linked genes B and S and the autosomal gene E^m. Apparently B interacting with S and E^m gives the melanophores of the male donor the capacity to produce

alternating white and black bars in the host feathers. The presence of *B* provides the melanophores with the special property of restricting the deposition of black pigment to bars in the contour feathers. The correctness of this interpretation is indicated by three lines of evidence. (1) Melanophores from the female hybrid donor and from donors of breeds such as the New Hampshire Red, Black and Buff Minorca, where the gene for barring is absent, all produce a non-barred coloration in contour feathers of the host. (2) Melanophores from male and female Barred Plymouth Rock donors produce differences in pattern. The melanophore from a female which has only one gene for barring produces a black bar of greater width than one from a male which has two genes for barring. (3) Melanophores from donors of non-barred breeds (New Hampshire Red, Buff and Black Minorca) do not produce a barred pattern in the Barred Rock host. Clearly then the capacity to produce barring depends upon the bar gene in the melanophore (see WILLIER 1941, p. 138 and WILLIER and RAWLES 1940, p. 191).

Although *B*, *S* and E^m are requisite factors in the melanophore for the production of black and white barring, the specific pattern produced is under the control of the feather germ of the host. This is shown by the wide variety of barred patterns produced in the same host by melanophores all of which are derived from the head region of a single donor embryo. The feather may be wholly black except for white bars on the shaft (*viz.*, P_1, P_2, and P_3 and in S_2, S_3 and S_4) or distinctly barred throughout the entire vane and shaft (*viz.*, S_{11}, S_{12} and S_{13}, breast, wing coverts). Between these two extremes within the flight series are many intermediate patterns in pigmentation. The vane of the feather may be (1) barred at the apex and non-barred basally and vice versa, (2) barred at both apex and base with the intervening portion non-barred or faintly barred and (3) distinctly barred in the inner half while the outer half is faintly barred or uniformly black. The relative amount of barred and non-barred portions of the vane varies from feather to feather within the series. The feathers showing intermediate pigmentation patterns constitute a perfectly graded series ranging from non-barred to barred types, roughly paralleling the order of emergence of the feathers. Furthermore, the width of the bars varies from feather to feather especially if they are in different tracts. The black bar may be very wide and the white bar relatively narrow (flight feathers). In others (breast) the white bar may be wide and the black bar relatively narrow or the white and black bars may be of nearly equal width (certain covert and secondary flight feathers). It is thus apparent that the individual feather germs vary greatly in their capacity to control the particular quality of barred pattern produced by the melanophores. Each one exercises a specifically different mode of control. The kind of barred pattern actually formed varies with the position of the feather, not only within a tract but from tract to tract. Regional differences in the pigmentation of the feather vane indicate that there are corresponding regional differences of control within the developing feather germ, one region provided with conditions favorable for barring and another for a somewhat uniform black pigmentation.

It is clear from the above considerations that the formation of the barred

pigmentation pattern must involve the interaction of the melanophores and the feather germ. The relative extent to which each component influences the formation of barred pattern is the problem now before us. It was argued in a previous paper from evidence then available (for details see WILLIER 1941) that the feather germ possesses a rhythm in physiological activity to which only the barred melanophores react. The failure of the melanophores of non-barred breeds to respond to the rhythm (even when introduced into a Barred Rock host) was attributed to a difference in threshold of reaction in melanophores from barred and non-barred varieties. This hypothesis involves a number of difficult assumptions, chief of which is the generalization which necessarily follows—namely, that the feather germs of all of the domestic breeds of fowl tested have fundamental rhythms in common. Another is that the rhythm must be present in one part of the feather germ and not in another (as for example, in feathers where the apex is barred and the proximal portion non-barred, or where one vane half is barred and the other half non-barred). Also in feathers where the barring is not synchronous in the vane halves, the rhythms in the two halves of the feather germ would have to be independent of each other.

New light has been thrown on this problem by recent investigations carried out in this laboratory by NICKERSON (1944) the primary purpose of which was to determine more precisely the respective rôles that the melanophore and the feather germ play in the production of rhythmic pigment bars. Inasmuch as the black and white barred patterns in the Silver Campine and the Barred Plymouth Rock are genetically and structurally distinctive, these breeds were selected for the analysis. From measurements of growth rate and bar width NICKERSON calculated the time required to form a complete bar (black +white) in a normal regenerating feather of each breed. The time period for barring was found to be distinctly different—that is, significantly shorter in the Campine than in the Barred Rock. Furthermore, when melanophores from these breeds were introduced into White Leghorn feathers by several grafting methods, the time period specific to the donor was invariably produced in the host feathers. On the basis of the assumption that the Campine and Rock melanophores reacted specifically to rhythmic processes in the feather germ, these results would require the existence of at least two independent time rhythms in the same feather germ of the White Leghorn host. Such a view is hardly conceivable. It is evident, therefore, that the control of the barring rhythm resides primarily in the melanophores themselves rather than in the host feather germs.[2]

[2] On the basis of results obtained, NICKERSON (1944) postulated that the barring rhythm is controlled primarily through the medium of diffusible substances which are produced by the melanophores within the black bar and inhibit pigment formation in the precursor melanophores present in the barb ridges of the subjacent white bar of a developing feather. Certain properties of the feather germ, such as growth rate, barb ridge size, etc. are likewise involved. These are conceived of as modifying the rate of diffusion and concentration of the inhibitor substance in the zone of differentiation of the melanophores (a narrow transverse zone intersecting the barb

What is the nature of the influence of the feather germ in the production of the barring pattern? That a relationship exists between the growth rate of individual feather germs and the specific quality of barred pattern formed by the F_1 hybrid male melanophores is indicated by several lines of evidence. Measurements of the rate of elongation of each juvenile flight feather of control White Leghorn chicks show that there is considerable difference between the late emerging secondaries and the early emerging primaries. Two examples will be used to illustrate the difference. In a female chick P_1 and P_2 elongated at the respective rates of 3.14 and 2.97 mm per day while S9 and S10 increased in length at the respective rates of 2.23 and 2.24 mm per day. In corresponding donor colored feathers of a female host it is found that the primaries are uniformly black except for some white on the proximal half of the shaft while the secondaries are distinctly barred at apex and base, the intervening portion being indistinctly barred. In a male control White Leghorn chick, P_2, P_3 and P_4 elongated at the respective rates of 2.17, 2.17 and 2.14 mm per day while S9, S10 and S11 elongated at the respective rates of 1.91, 1.91 and 1.89 mm per day. In a male host, the corresponding donor-colored primaries are uniformly black except that P_4 has a white bar at the base of the inner vane half—in all three the shaft exhibits alternating bands of black and white. The corresponding secondaries have a barred pattern which is most distinctive in S11 (the slowest growing one). These observations indicate that the conditions for the production of a barred pattern are more favorable in slow growing feather papillae than in rapidly growing ones. Growth rate appears to be a modifying factor affecting the rhythmic production of pigment by the melanophores.

In this connection it is of interest to compare the feather color patterns produced in the White Leghorn host chick with those of homologous feathers in the donor chick which had furnished the melanophores. Two pairs of such combinations hatched and lived until after the juvenile contour plumage had completely emerged. Although the melanophores acting in both host and donor are genotypically the same, the pigmentation patterns are quite different. In the host the primaries are uniformly black and not barred; the axillary and most of the secondaries (S1 to S11) are more or less uniformly black with faint indications of white bars on the shaft and sometimes with a single white bar across the tip of the vane; and the remaining secondaries (S12–S14) as well as the upper and lower wing coverts are distinctly barred. In the donor control the corresponding flight feathers and coverts are all barred. The quality of the barred pattern varies, however, from feather to feather and in the axillary, S1, P1 and P2 it is mostly confined to the shaft and vane tip. These differences in pattern produced by the same melanophores may be attributed to differences in rate of feathering, the host being rapid feathering and the hybrid relatively

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ridges at a level adjacent and apical to the collar). The modification thus produced may be a specific one in a given feather germ inasmuch as growth rate and barb ridge size vary more or less specifically in the different feather germs. In this way the variation in quality of barred pattern from feather to feather may be explained.

slow feathering. Although the rates of growth of the wing feathers in host and donor were unfortunately not determined, evidence in general from our measurements of the rate of elongation of flight feathers in several breeds of fowl, indicate that it is higher in the White Leghorn than in other breeds such as the B. P. Rock and R. I. Red (see WILLIER and RAWLES 1940, fig. 1, p. 184).

Although rate of elongation thus appears to be a factor in determining the quality of barred pattern produced it appears not to be the sole one. In one female host, P8 is practically uniformly black and S10 is definitely barred. In one control White Leghorn female, P8 grew at the rate of 1.98 mm per day while S10 grew a little more rapidly—at the rate of 2.24 mm per day. Also in one male host S1 is uniformly black while S11 is distinctly barred. In the male control White Leghorn, S1 and S11 grew at the respective rates of 1.76 and 1.89 mm per day. In these two cases the feathers having the lower rate are non-barred which is just the reverse of the usual finding. Furthermore, if barring is to be correlated with a slow rate of growth and non-barring with a relatively higher rate, a difference in the quality of barring should be found in flight feathers having a marked asymmetry, where according to MONTALENTI (*l.c.*) the rate of barb formation is higher in the longer vane side than in the short vane side. This is not borne out by the evidence, since P6 and P7 have a barred pattern in each vane side irrespective of its width. This appears to be generally the case throughout the flight series of feathers. In the control F1 hybrid males P6 and P7 (juvenile contour plumage) show the same situation, but in P4 and P5 the inner vane (long side) is barred while the outer vane (short side) is non-barred. In view of such observations as these it is evident that physiological factors other than growth rates also must be concerned. For the probable general nature of these the reader is referred to p. 321. Still other conditions which must be taken into account are (1) the nutritional conditions of the chick during feather formation and pigmentation and (2) the genetic variations in rate of feathering within the group of experimental and control birds examined.

SUMMARY

1. This investigation deals with the control of feather pigmentation pattern as revealed by grafting to White Leghorn embryos precursor melanophores from hybrid embryos (R.I. Red♂×B.P. Rock♀), which after hatching show sex-linked differences in coloration of the plumage. The donor and host embryos usually were approximately of the same age.

2. The source of the melanophores was skin ectoderm from the head, the wing and leg bud and also pure mesoderm from the head and the wing bud. A small piece of the donor tissue was inserted into the mesoderm at the base of the right wing bud of the host. In 16 cases the sex of the donor furnishing the melanophores was determined. Two donor-host pairs hatched and lived which made it possible to compare the feather color pattern produced in the host with that of the donor which furnished the melanophores.

3. The melanoblasts migrated out from the grafted tissue and produced an area of black-colored down feathers on the host at and about the site of graft-

ing, often extending over most of the wing and occasionally to the breast and shoulder.

4. After hatching the donor-colored down feathers were gradually and normally replaced by contour feathers of the juvenile plumage. In every case in which the sex of the donor was ascertained, the color pattern produced in host contour feathers was in accordance with the sex-linked differences in the plumage coloration of control chicks.

The melanophores of the male which are heterozygous for the sex-linked genes *B* and *S* and the autosomal gene E^m (*Bb Ss*; $E^m e^m$) produce a black and white barred pattern—that is, not only *B* but also *S* and E^m express themselves in the melanophores. On the other hand, the melanophores of the female which are genotypically non-barred and non-silver (*bs*; $E^m e^m$) produce a non-barred pigmentation. Thus the primary controlling factor in color pattern formation is the genotypic constitution of the melanophore.

5. The specific pigmentation pattern produced by either the male or female melanophores depends upon the individual feather germs, varying with the time of emergence and the position of the feather on the body of the host. Each feather germ produces its own particular color pattern. The sex of the host has no influence on the pattern produced by the grafted melanophores.

6. The variations in the character of the non-barred pigmentation produced appear to depend primarily upon differences in the expressivity of E^m in the melanophores of the various female donors—that is, whether all of the pigmented feathers of a particular host will have either (a) a uniformly black coloration almost without exception or (b) a black-red ("stippled") pattern. From such differences in phenotypic expression it is inferred that the precursor melanophores from the different female donors have different potencies—that is, either a potency for differentiating usually only black melanophores (E^m fully expressed or nearly so) or a potency for differentiating either a red or a black melanophore (expression of E^m sufficiently weakened by modifying factors). In the latter case whether the black or red potency is realized depends apparently upon the physiological properties characteristic of the individual feather germs of the host. Of these properties, the reaction gradient in the epidermal substratum, which differs in value and direction in the different feather germs, appears to play a rôle in the differentiation of the kind of pigment cell. Growth rate of the feather germ must also be a factor since in general a high rate favors the formation of black melanophores while a relatively slower rate favors the formation of red melanophores.

7. The presence of *B*, *S* and E^m are requisite factors in the melanophore of the male hybrid for the formation of black and white barred pattern. *B* provides the melanophore with the special property of behaving rhythmically in pigment production. The rhythmic production of pigment by the melanophores, however, is modified by physiological properties characteristic of the feather germ. Growth rate of the feather germ appears to be one of the modifying factors, since in general a correlation exists between relatively slow rate and distinct barring and between relatively high rate and indistinct barring or its absence. Factors other than growth rate are also involved.

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HETEROSIS IN COMPOUNDS OF LOZENGE ALLELES IN DROSOPHILA MELANOGASTER

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IN HIS book, "Genetics and the Origin of Species," DOBZHANSKY referred to the difficulty encountered in measuring true mutation rates due to lack of knowledge regarding the manifold effects of genes. A person who first describes a mutant usually stresses the easily detectable and striking characteristics of the mutant and leaves the description of the multiple effects of the gene to the person whose problem requires it.

Comparative studies of a series of alleles offer one method of determining more completely the manifold effects of a gene. It is true, however, that the method which has been used in the selection of mutants, a selection based upon the first described and striking characteristics associated with a gene, may have failed to detect some of the possible alleles for a given locus. The occurrences of mutations at the white locus in *Drosophila melanogaster* have been detected by variation in eye pigment. However, DOBZHANSKY (1927) and DOBZHANSKY and HOLZ (1943) have shown that the gene for white eye (and also some other genes associated with pigment development) may affect internal structures which seem totally unrelated to the traits generally ascribed to the gene. An analogous condition is found with the gene for lozenge eye. As shown by the known alleles, the gene affects several characters. The lozenge effects are observed in the altered size and shape of the eye, the arrangement of the facets, the pigment of the eye including its distribution (GOTTSCHEWSKI 1936; OLIVER unpublished) and the fertility of the females. An effect of the lozenge gene on the internal genitalia of females has been reported for the two lozenge alleles glossy and spectacle (OLIVER and GREEN 1939; GREEN and OLIVER 1941). A like effect in the original lozenge mutant is reported in this communication, and the effect in several other alleles will be reported more fully by R. C. ANDERSON (thesis). It is definitely possible that some alleles of lozenge (or of white) may affect only the internal genitalia, and because they have no effect on the eyes may have escaped detection. Even though all the alleles of a locus may not be known, a knowledge of the nature of a gene and of its action during development may be gained by a study of known alleles of that gene.

We have been studying the manifold effects of the lozenge locus as shown by the morphological and physiological characteristics associated with the known alleles and by their interactions with other genes. In his report on 14 lozenge alleles, GOTTSCHEWSKI concluded that the series can be divided into three groups, with one group including only almondex, and that the interactions within and between the groups differed. In our studies, to be communicated later, we do not find conditions to be that simple. Two alleles may look ex-

actly alike as generally measured and yet differ with respect to dominance over their alleles belonging in the other group and even with respect to their dominance over the normal allele.

One of the actions of the lozenge gene is observed as a decreased vigor of the homozygous females. This communication reports a study of the viability, fertility, and fecundity of three of the alleles.

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MATERIALS AND METHODS

Three alleles, glossy, spectacle and lozenge, were used in a comparative study of viability, fertility, and fecundity. All three are recessive to the normal allele. Glossy (*lz^g*) has been described as an X-ray-induced mutant with a blood-red eye color, with the eye almost normal in shape and size and with the arrangement of the facets causing the eye to have a wettish, glossy appearance (OLIVER 1940). Glossy is located in the *dl-49* inversion of the X chromosome. Spectacle (*lz^s*), also in the *dl-49* inversion, causes the eyes to have smooth facets and a light-brown color with a heavily pigmented rim. It has been described by PATTERSON and MULLER (1930). Lozenge (*lz*), which was received from C. B. BRIDGES, has the typical shape and structure of the eye descriptive of the mutant. The three mutants are easily distinguishable with respect to the characteristics of the eye.

Compounds of the three alleles differ in their expression of the pigment, shape, and facet-structure of the eye. Although *lz^g/lz^s* females resemble homozygous *lz^g* females, each of the other two compounds is distinguishable from the homozygous females. Except for the similarity between *lz^g/lz^s* and *lz^g/lz^g*, one encounters no difficulty in determining by observation of the eyes which homozygous or compound female he is studying.

As controls, the *lz$^+$* (normal) which had been obtained by crossing over between *lz^s* and *lz^g* was used (OLIVER 1940). Females that are *lz^s/lz^g* should when mated to *lz^g* males produce spectacle (*lz^s*) sons, glossy (*lz^g*) sons, and glossy-like (*lz^g/lz^g* or *lz^s/lz^g*) daughters. Among 8393 offspring produced by that parental combination, however, 14 occurred that were normal (*lz$^+$*) for all the eye and genital characters known to be abnormal in lozenge flies. The *lz$^+$* is dominant to both *lz^s* and *lz^g*.

In every test in which mutant genes were used as markers to the left and right of the lozenge locus, the reversion to *lz$^+$* occurred in association with crossing over, and that crossover type had the genes which were to the right of *lz^g* in the *lz^g*-bearing chromosome and those to the left of *lz^s* in the *lz^s*-bearing chromosome. Since both *lz^s* and *lz^g* are in the *dl-49* inversion, the loci *y Hw m* and *v* are to the left of the lozenge locus and *sn vs f* and *Bx* are to the right. (The symbols represent yellow, Hairy-wing, miniature, vermilion, singed, vesiculated, forked and Beadex, and their respective loci in the non-inverted genic map are 0, 0+, 36.1, 33, 21, 16.3, 56.7, and 59.4, with lozenge being lo-

cated at 27.7.) Females *lz*^{s} *f*/*lz*^{g} *Bx*^{2} produced eight *lz*$^{+}$ *Bx*^{2} and one *lz*$^{+}$ *f*, the latter probably a double crossover. Females *y Hw lz*^{s}/*lz*^{g} *Bx*^{2} produced two *y Hw lz*$^{+}$ *Bx*^{2} flies. Two *v lz*$^{+}$ *Bx*^{2} males occurred among the offspring of *v lz*^{s} *f*/*m*^{2} *lz*^{g} *Bx*^{2} females. One *m*^{2} *lz*$^{+}$ *vs*^{28} fly was produced by *m*^{2} *lz*^{s} *f*/*v lz*^{g} *vs*^{28} females, although the presence of vesiculated could be determined only by tests. Subsequent to the frequency studies, three *y Hw lz*$^{+}$ *sn*^{41} *vs*^{28} males have been observed and used from among the uncounted progeny of *y Hw lz*^{s}/*v lz*^{g} *sn*^{41} *vs*^{28} females. Where markers have been used, the *lz*$^{+}$ reverion has always been associated with crossovers which have occurred between the genes closest to the lozenge locus. Apparently the crossing over has occurred between the two alleles *lz*^{s} and *lz*^{g}.

It is not possible as yet to state whether the *lz*$^{+}$ crossover type represents another case of unequal crossing over similar to that known with the Bar locus (STURTEVANT 1925) and with the Star locus (LEWIS 1941). If the complementary crossover type occurs, every genetic test so far used has failed to detect it. Cytological examinations of the salivary chromosomes of *lz*$^{+}$ indicate that the bands are identical to those in the *dl-49* inversion. One cytological observation is of interest. In some of the females heterozygous for *lz*^{s}, some but not all of the cells of the salivary glands show a torsion of one heavy band which is suggestive of a small inversion or possibly a duplication. (DR. A. B. GRIFFIN of TEXAS UNIVERSITY has been kind enough to check the slides with the senior author and has reported that the anomaly is in region 8 of the salivary chromosome map.) However, the twisting is not constant. Although it may be observed in some of the cells of *lz*^{s}/*lz*^{g} and *lz*^{s}/*lz*$^{+}$, it has not been detected in either *lz*^{g} or *lz*$^{+}$ without *lz*^{s}. The significance of the possible anomaly is not understood. Cytological and genetic examinations are to be continued.

Genotypes of the females used in the present study were *ClB*/*dl-49 lz*^{g} *Bx*^{2}, *ClB*/*dl-49 lz*^{s} *f*, *ClB*/*lz*, and *dl-49 lz*$^{+}$ *Bx*^{2}. The first three cultures were developed in 1938 from the mating of the respective males to *ClB*/*sb* females. Subsequent to the introduction of the genes *f* and *Bx*^{2} into the combinations, no attempt was made to get the cultures isogenic. The *dl-49 lz*$^{+}$ *Bx*^{2} culture was derived from a crossover male (in 1939) among the progeny of a *lz*^{s} *f*/*lz*^{g} *Bx*^{2} female. The *lz*$^{+}$ *Bx*^{2} male was mated to *ClB*/*sb*, and from that mating a homozygous culture was prepared. For purposes of simplification, the cultures will be referred to in this report as *lz*^{g}, *lz*^{s}, *lz*, and control, respectively.

In the test matings, virgin homozygous *lz*^{g}, *lz*^{s}, *lz* and control females and virgin compounds of *lz*^{g}/*lz*^{s}, *lz*^{g}/*lz*, and *lz*^{s}/*lz* females were mated individually to *lz*^{g} males in vials containing the banana-agar culture media. The matings were placed in a constant temperature chamber kept at 23 ± 1°C. On the fourth day after the mating, each vial was inspected. If the male parent had died, that culture was discarded from the test. If the female parent was dead, the culture was recorded but not used in subsequent counts. The proportion of mated females which were alive on the fourth day was used as a measure of the viability of the females. On the eighth day, parents in those cultures which had been alive on the fourth day were killed and the cultures were inspected

through the sides of the glass vials for evidences of larvae. Counts of offspring were made up to the eighteenth day after the matings. If dark pupae were observed in the vials on that day, the counts were continued through two more days. As a check against the possible retention of parents, the flies were etherized and counted even though only one fly could be observed in the vial. The proportion of those cultures which were alive on the fourth day and produced at least one offspring was used as a measure of the fertility of the females. The average number of offspring produced in the fertile cultures measured the fecundity of the females.

It was necessary to run numerous series of tests. After the first series, attempts were made to have equal numbers of fertile cultures of all the various types of females in each series; but due to the poor viability of *lz*^{s} and *lz* females in the test and stock cultures, it was not always possible to get equal numbers. Virgin females were mated within forty-eight hours after being collected, even though only a few of some types were available. Except for the first three series of tests, controls were tested concurrently with the mutant lozenge-allele females.

Because of an observation that *v lz*^{s} *f* (vermilion spectacle forked) females were more viable than *lz*^{s} females, comparative tests were run with *v lz*^{s} *f*, *v lz*^{g} *f*, and their compound *v lz*^{s} *f*/*v lz*^{g} *f*. The genotypes were made by mating *dl-49 lz*^{g} *Bx*^{2} and *dl-49 lz*^{s} to *dl-49 v f*, selecting males with the required combinations from the crossovers, and balancing each combination to the inversion *ClB*. The procedure does not build isogenic cultures, but it does give approximately identical X chromosomes except possibly for the region around the *lz* allele. Test matings were run in a manner identical to that already described. It had become necessary to use cornmeal culture media and a room with temperature control set at 72 ± 2.0°F. Consequently, the data cannot be compared to those of the earlier tests, but the results observed with the homozygous females can be compared to those obtained with their compound.

References to the methods used in egg-hatch counts will be made with the discussion of the observed results.

EXPERIMENTAL RESULTS

The data on viability, fertility, and fecundity are recorded in table 1. The first column gives the symbols to represent the genotypes of the females used. Symbol *lz*^{g} represents *dl-49 lz*^{g} *Bx*^{2}, and *lz*^{s} represents *dl-49 lz*^{s} *f*. Control females were *dl-49 Bx*^{2}, the reversion type resulting from crossing over between *lz*^{g} and *lz*^{s}. Each female had been mated to a *dl-49 lz*^{g} *Bx*^{2} male. The second column gives the number of matings made with each type of female. The column marked "viability" shows the number of the mated females of each type which were alive on the fourth day, the percentage each number represents of the mated females and the standard error of that proportion. The column marked "fertility" shows for each type the number of viable females which produced offspring, the proportion of the viable females which were fertile, and the standard error of the proportion. In the column under "fecun-

dity" are recorded the total number of offspring produced by fertile females of each type, the mean, standard error of the mean, and the standard deviation.

Comparison of homozygous females

Homozygous lz^g females were the most viable and lz^s females the least viable of the three alleles. The lz^s females, however, were more fertile than lz^g females, at least as fertile as *lz* females, and more fecund than either lz^g or *lz* females. Significant differences were found in the viability tests for the homozygous females. Of the mated females, 83.1 percent of lz^g, 40.3 percent of *lz*,

TABLE 1

Viability, fertility, and fecundity of lz alleles.

♀ TYPE	NO. ♀ MATED	VIABILITY			FERTILITY			FECUNDITY			
		NO.	%	S.E.	NO.	%	S.E.	NO. F_1	M	S.E.M.	S.D.
lz^g	899	747	83.1	1.25	173	23.2	1.54	1616	9.3	0.79	10.4
lz^s	496	142	28.6	2.03	50	35.2	4.01	755	15.1	1.77	12.5
lz	523	211	40.3	2.14	61	28.9	3.12	482	7.9	1.21	9.5
lz^g/lz^s	468	430	91.9	1.26	209	48.6	2.41	5503	26.3	1.34	19.4
lz^g/lz	455	371	81.5	1.82	131	35.3	2.48	2775	21.2	1.58	18.1
lz^s/lz	196	165	84.2	2.61	79	47.9	3.89	1966	24.9	2.28	20.4
Control	135	122	90.4	2.54	105	86.0	3.14	6351	60.5	2.02	20.7

Each ♀ was mated to lz^g ♂ in a vial. The methods and the genotypes of the females are given in the text. Viability is measured by the percentage of females which were alive on the fourth day after the mating. Fertility is based upon the proportion of living females which produced at least one offspring. Fecundity is measured by the average number of offspring produced by the fertile females.

and 28.6 percent of lz^s females were alive on the fourth day. The difference between females *lz* and lz^s which were closest in the degree of viability is approximately four times the standard error of the difference. Among those which lived four days, only a relatively small proportion of the females of either type produced any offspring. The comparative proportions of producing females for the three alleles were not extremely different. Only lz^s with 35.2 percent and lz^g with 23.2 percent of the females being fertile were significantly different. Their difference is 2.8 times the standard error of the difference. The observed proportion of fertile *lz* females, 28.9 percent, falls within the limits of chance variation when compared to lz^s or lz^g. Even those females which were fertile produced on the average only a small number of offspring. Females lz^s produced an average of 15.1 offspring and were the most fecund; *lz* females with a mean of 7.9 offspring were the least fecund. In comparison with either lz^g or *lz*, the lz^s females produced a significantly higher average number of offspring, the D/SED being 2.99 and 3.36, respectively. Females lz^g, with an average of 9.3 offspring per fertile female, differed with *lz* only to the extent of the standard error of the difference.

In comparison with the control females, all three females homozygous for the lozenge alleles were significantly less viable, fertile, and fecund. Although 83.1 percent of the lz^g females were alive on the fourth day, 90.4 percent of the control females were alive. The difference between the two is 2.6 times the standard error of the difference and cannot be considered due to chance alone. In the viability tests of lz^s and lz and in the fertility and fecundity tests of all three alleles, the data in table 1 clearly show that the three homozygous alleles with the genotypes used in the tests were significantly less vigorous than the control females.

Comparison of compounds

Among the three compounds, lz^g/lz^s showed the highest and lz^g/lz the lowest viability, fertility, and fecundity. Compound lz^g/lz^s females with 91.9 percent of the females living four days had a significantly higher viability than either lz^s/lz or lz^g/lz. The difference between lz^s/lz and lz^g/lz, with 84.2 and 81.5 of their respective females living four days, was not so great as the standard error of the difference and may be considered to be due to chance. In the fertility tests, compounds lz^g/lz^s and lz^s/lz showed 48.6 percent and 47.9 percent of the viable females to be fertile. Although the difference between the two compounds is not significant, each of the types differs significantly from lz^g/lz which had only 35.3 percent of the living females producing offspring. The respective differences are found to be 3.8 and 2.7 times the standard error of the differences. Only the most fecund compound, lz^g/lz^s which produced an average of 26.3 offspring, and the least fecund compound, lz^g/lz with a mean of 21.2 offspring, may be considered to have a true difference in fecundity. The difference between the means is 2.5 times the standard error of the difference. The mean number of offspring produced by lz^s/lz, 24.9, does not differ significantly from that of either of the other compounds.

Although the results show that lz^g/lz^s females were more viable and lz^s/lz females were less viable than control females, the observed difference between the controls and either one of the two compounds is not significant. However, the difference in viability between the controls and lz^g/lz is 2.9 times the standard error of the difference and is apparently due to an actual viability difference. With respect to fertility and fecundity, very significant differences were observed between control females and even the most fertile and most fecund of the compound females. In comparison with the results obtained with the control females, the compound females were from 90 to 102 percent as viable, 41 to 57 percent as fertile, and 35 to 43 percent as fecund as the control females.

Comparison of compounds and homozygotes

With but two exceptions in the nine tests, each compound of the lozenge alleles proved to be more vigorous than the homozygous females used in the compound. Only lz^g/lz, the compound with the lowest recorded viability, fertility, and fecundity, failed to show more vigor than the homozygous alleles used to form the compound. Actually a slightly smaller proportion of the lz^g/lz females than of the homozygous lz^g females was alive on the fourth day, but

the difference is less than the standard error of the difference, the D/SED being 0.7; consequently, the two types of females may be considered to have equal viability. Homozygous *lz* females, the other allele used in the compound, were significantly less viable than the lz^g/lz females (the D/SED being 14.7). A larger proportion of the compound females than lz^g females produced offspring, and the D/SED value (4.1) indicates that the difference in vigor associated with increased fertility is significant. However, the difference in fertility between the compound and *lz* females has a D/SED value of only 1.6 and cannot be considered significant. In the tests of fecundity, lz^g/lz females were significantly more productive than either lz^g or *lz* females, the D/SED being 6.7 in each case. The other two compounds, lz^g/lz^s and lz^s/lz were significantly more vigorous in all three tests than were the respective alleles in homozygous females. Comparisons of the differences show that D/SED values range from 2.3 to 26.5.

Some of the factors associated with the increase in fecundity of the compound females may be shown by grouping the fertile cultures into classes showing the number of offspring produced. Among the 173 fertile lz^g females, for example, there were 38 which produced only one offspring, 12 which produced two, 16 which produced three, 14 which produced four, and so on. Approximately one-half of the lz^g females produced fewer than five offspring. The same general condition was found with lz^s and *lz* females.

The grouped data on fecundity are represented by histograms in figure 1. The base lines of the graphs represent the number of progeny per culture. In order to reduce the number of zero groups, the cultures were grouped into classes with boundaries of four—that is, all cultures having at least one and fewer than five offspring were grouped into one class; all with at least five but fewer than nine, into the second class; and so on. The vertical lines represent the proportions of fertile cultures which belong to the various classes. The graphs for the homozygous females of all three alleles show that a high proportion of the cultures produced only a few offspring, although an occasional female did produce a relatively large number of progeny. The most fecund lz^g female produced 54 offspring. One of the 50 lz^s females produced 52, and one of the 61 *lz* females produced 54 offspring. As expressed by the graphs, many of the compound females also produced only a few offspring. However, as compared to homozygous females, a greater proportion of the compound females tended to fall into the more fecund groups; moreover, the most fecund females produced more offspring than did the highest producers of the homozygous females. Control females varied in fecundity, producing from 1 to 106 offspring per culture, and very few of the females were found to fall into the classes with low fecundity. The extreme skewness observed in the graphs of the homozygous and compound females is not seen with the control females.

Modifiers affecting heterosis

It seemed possible that the heterosis observed in the compounds might be due in part, at least, to modifying genes. Consequently, partially isogenic cultures were made, as described under methods. The results are not exactly com-

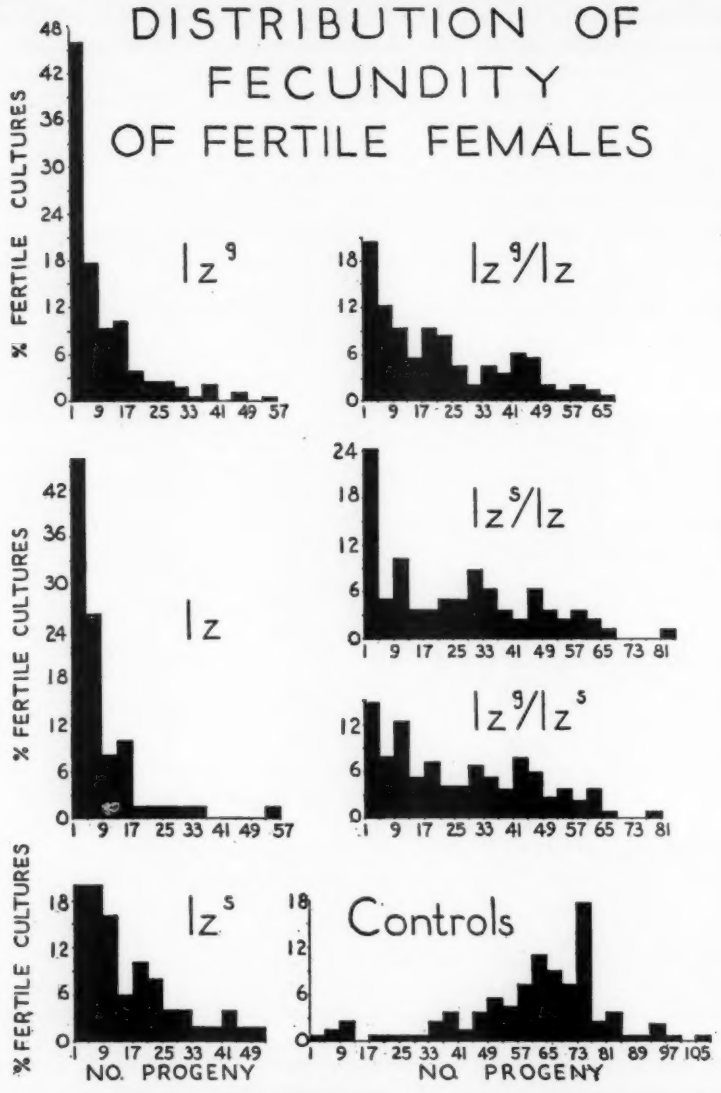

FIG. 1.—Distribution of fecundity of fertile females. The three homozygous females are represented on the left side; the three compound and the control females, on the right. The base lines show the ranges of fecundity. Cultures are distributed into groups with class boundaries of four. The vertical lines show the proportion of fertile cultures that belong to each class. For total number of fertile cultures, see table 1.

parable to the other tests of the alleles, since they were not run concurrently and were made under different media and temperature conditions (see under methods). However, the results are of interest and merit some discussion.

Data on the tests with *v lz^g f* and *v lz^s f* females and their compound are given in table 2. Homozygous *v lz^g f* females were more viable, fertile, and fecund than *v lz^s f* females. Their compound *v lz^g f/v lz^s f* showed heterosis in each test. If the results of the tests of the partially isogenic cultures are compared to those of the non-isogenic cultures (tables 1 and 2), the data show that *v lz^g f* females were equally viable as and more fertile and fecund than the *lz^g* females. The *v lz^s f* females were more viable and fertile but not significantly more fecund than the *l^s* females. In the compounds, *v lz^g f/v lz^s f* equalled the viability observed in *lz^g/lz^s* and had an increased fecundity and fertility.

TABLE 2

Viability, fertility, and fecundity of partially isogenic females.

GENOTYPE	NO. MATED	VIABILITY			FERTILITY			FECUNDITY			
		NO.	%	S.E.	NO.	%	S.E.	NO. F_1	M.	S.E.M.	S.D.
v lz^g f / *v lz^g f*	72	58	80.6	4.66	47	81.0	5.15	1873	39.8	3.54	24.3
v lz^s f / *v lz^s f*	71	38	53.5	5.92	22	57.9	8.01	488	22.2	4.64	22.2
v lz^g f / *v lz^s f*	54	50	92.6	3.55	48	96.0	2.77	2733	56.9	3.27	22.6

See table 1 for explanations of symbols.

Although, as stated, the two series of tests do not permit an accurate comparison, the data definitely indicate that modifying genes affect the viability, fertility, and fecundity of at least two *lz* alleles. However, heterosis between the alleles also occurs as shown by the significant increase in viability, fertility, and fecundity of the compound over the homozygous alleles of the partially isogenic cultures.

Morphological anomalies associated with the alleles

Homozygous *lz^g*, *lz^s*, *lz* females, their compounds, the isogenic cultures, and wild type females were dissected, and the internal genitalia were examined. The homozygous lozenge-allele females and the compound females lacked spermathecae and parovaria, a condition which has already been reported for *lz^g* and *lz^s* (OLIVER and GRÈEN 1939). Ovaries and oviducts in the lozenge-allele and in the compound females were not found to differ from those in normal females. Eggs were observed forming in the ovaries and passing through the

oviduct and uterus. Coiled ventral receptacles were observed in all females. It will be recalled that in *Drosophila melanogaster* the oviducts, spermathecae, parovaria, and ventral receptacle develop from a genital ridge of the larva and that the ovary which is present in the larva persists in the adult.

In preparations of the genitalia, the ventral receptacles were spread and the tissue stained with eosin and with fast-green. Examinations of the whole mounts indicated that the lumina of receptacles in lozenge-allele females were as long and as wide as the lumina in normal females. In some of the lozenge-allele females, a mass of material was observed in the lumen, but the same condition was found in some of the normal females. The significance of the mass material has been studied by R. C. ANDERSON and will be reported in another communication.

Eggs with chorion intact and with chorion removed were fixed in Kahles solution and stained with eosin and with acetocarmine. No differences between eggs from lozenge-allele females and those from normal females could be detected.

Egg-hatch count

Tests were made to determine whether the low fertility and fecundity of *lz*^{g} females were due to low egg production. Homozygous *lz*^{g} and control females were placed individually in vials with single wild type males. At the end of the day, the males were killed in each vial in which copulation had been observed. The females were transferred without etherization to a vial which contained a spoon with a small amount of food. At the end of 24 hours, new spoons were placed in the vials with the females. The eggs which had been deposited on the old spoon during the 24-hour period were counted, and the food with the eggs was transferred from the spoon to a vial of food. After five such transfers, the females were transferred to vials of food unless larvae had been observed in earlier containers.

The offspring which hatched in each vial, including the mating vial, were counted. In 13 of the 105 counts in which both eggs and offspring occurred, there were a few more offspring than counted eggs. The unfortunate mishap prevents an accurate estimate of the egg-hatch ratio, but it does not interfere with the main purpose of the study—that is, to determine whether there is any relation between the degree of fecundity and egg production.

Only 36 *lz*^{g} females and 16 control females were observed to mate, approximately 30 percent of each type of female tested. Twenty-two *lz*^{g} females and 15 control females produced eggs. Only eight of the 22 egg-producing *lz*^{g} females produced offspring as compared to 13 of the 15 control females.

The results of the samples are shown in table 3. The total number of females which produced eggs on each day is given in the first horizontal column. Females differed in the number of days in which they produced eggs. Four of the 22 *lz*^{g} females produced eggs on only one spoon, but eleven produced eggs on all five spoons. Two *lz*^{g} females produced eggs on two spoons; two on three; and three on four spoons. Nine of the 15 control females produced eggs five days. Four produced eggs on four spoons and two produced eggs on only two spoons.

TABLE 3
Daily egg and offspring production.

	lz^g					CONTROL					
	MATING VIAL	SPOONS					MATING VIAL	SPOONS				
		1	2	3	4	5		1	2	3	4	5
No. ♀ producing eggs per day	—	14	16	17	19	16	—	13	13	13	13	13
Av. no. eggs per daily producer	—	8	19	18	15	19	—	19	26	18	21	19
No. ♀ producing offspring per day	2	6	6	5	2	1	3	12	12	9	10	10
Av. no. F_1 per daily producer	2.5	3	7	2	2	2	16.5	19	24	13	10	6

Based upon a total of 22 egg-producers and eight breeders of *lz*^{g} females and 15 egg-producers and 13 breeders of control females. Egg counts were not made in mating vials.

The second horizontal column in the table shows the average number of eggs produced daily by the egg-producers of that day. Every female varied in her daily production. The greatest number of eggs counted for any *lz*^{g} or control female on one spoon was 48. Females also varied in the total number of eggs deposited during the productive period. Three of the *lz*^{g} females produced only one egg each. However, the most productive female deposited 184 eggs; and three of the 14 sterile and two of the eight fertile females produced more than 100 eggs each. The total number of eggs produced by the control females varied from 22 to 163. One of the two sterile and seven of 13 fertile females produced more than 100 eggs. The *lz*^{g} females were not as proficient egg-producers as were the control females. Only 61 percent of the former as compared to 94 percent of the latter produced eggs. In daily production, the proportion of producers varied from 39 to 53 percent for *lz*^{g} females, but on each day 81 percent of the mated control females produced eggs. As shown by the table, however, the average number of eggs deposited each day by the producing *lz*^{g} females was not much lower than the average number observed for the control females.

The number of females which produced offspring on each day is shown in the third horizontal column of table 3, and the mean number of progeny for each set of daily producers is given in the fourth column. During the last two daily counts, only a small number of *lz*^{g} females produced offspring, although they continued to produce eggs. On the other hand, most of the control females produced offspring as well as eggs throughout the test period. The *lz*^{g} and control females also differed in the mean number of offspring produced, although the two kinds of females deposited approximately the same average number of eggs. In both of the female types, however, the greatest number of fertile eggs occurred on the second day and the proportion diminished toward the end of the test, but the decrease in the proportion of fertilized eggs occurred more rapidly with the *lz*^{g} females.

The number of offspring produced by *lz*^{g} females ranged from two to 27, but only two of the eight breeders produced more than eight offspring. No constant relationship was found between the number of eggs deposited and the number of offspring produced by a female. For instance, one *lz*^{g} female deposited 184 eggs during the five-day test, but she produced only three offspring, and they appeared from the eggs on the first spoon. One female produced two offspring in the pre-test vial (mating vial) and 19 eggs but no offspring on the first four spoons. The single *lz*^{g} female which produced two offspring on the last day of the test deposited no eggs on the first spoon, but had 118 eggs on the last four spoons. She produced a total of 16 offspring in the last four days with ten of them occurring from 23 eggs of the second spoon. Control females produced from 9 to 133 offspring. There was also no constant relationship between the number of eggs deposited and the number of offspring produced by the control females.

As shown by the number of fertile females per day, not every fertile female in either test produced offspring each day. Actually some produced only one day, some skipped one or more days, and others did not begin production until one or two days after mating. Most of the offspring produced by the *lz*^{g} females occurred during the first two of their productive days, a condition which is in sharp contrast to that found with the control females. Apparently, therefore, lack of egg production is not the main factor in decreased fecundity. For some reason, most of the eggs in *lz*^{g} females were not fertilized, even though copulation occurred. Those findings were in agreement with observations in the fertility and fecundity tests in which eggs were seen in sterile cultures and in vials in which only one or two offspring were counted.

DISCUSSION AND CONCLUSIONS

Three alleles of the lozenge locus and their compounds were tested for viability, fertility, and fecundity. As controls, females were used which were *lz*$^{+}$ (normal) as a consequence of crossing over between *lz*^{g} and *lz*^{s}. Viability was measured by the proportion of females which lived four days after the mating; fertility, by the proportion of living females which produced offspring; and fecundity, by the average number of offspring produced by the fertile females.

Significant differences among the homozygous females were found in the viability tests of the three alleles. However, only *lz*^{s} and *lz*^{g} differed significantly in fertility, and only *lz*^{s} can be considered to be more fecund than either of the other alleles.

The three homozygous alleles were low in viability, fertility, and fecundity when compared with control females. Homozygous *lz*^{g} was 92 percent as viable as control females, but the other two types were less than 50 percent as viable. All three alleles were less than 42 percent as fertile and less than 25 percent as fecund as the control females. Many of the lozenge-allele females were sterile, and most of those which did produce offspring were very infertile. The most fecund females produced fewer offspring than the average number produced by the control females.

Factors related to low vigor of homozygous females

The causal factors associated with the low vigor of the homozygous females can be determined only in part. No methods to measure the cause of decreased viability are available, but the data do offer some suggestions regarding fertility and fecundity. A smaller proportion of the lz^g females produced eggs during the five-day period than did control females, 61 percent as compared to 94 percent in the small numbers tested. Yet it seems very probable that most of the lz^g females are capable of producing eggs and that the non-productive ones behave more as virgin females which tend to hold their eggs. In the egg-hatch tests of this study the females had copulated and egg production was stimulated in some of them. Apparently all lz^g, lz^s, and lz females have normal ovaries and oviducts. Eggs can be observed in the oviduct or uterus of sterile females as well as in many of the vials containing females of low fecundity. Under microscopic examination, the eggs could not be differentiated from eggs produced by wild type females. As shown by the average daily egg counts, those lz^g females which deposited eggs were almost as productive as control females.

A lack of egg production does not seem to be the main factor in causing the low fertility and fecundity. Homozygous lozenge-allele females tend to produce fewer offspring than eggs. One lz^g female produced 118 eggs but only 16 offspring. Moreover, the offspring which are produced by either of the three alleles tested tend to occur within two or, occasionally, three days after insemination, even though the female produces eggs after that. Apparently, most of the eggs deposited by the homozygous lozenge-allele females are not fertilized. After the second day, in the tests made with lz^g, there was a rapid decline in number of daily offspring producers and in mean number of offspring in contrast to the conditions found in the controls. It seems that the phenomena are related to the inability of the sperm to retain the power to fertilize the eggs, probably due to non-motility of the sperm (OLIVER and ANDERSON 1943; ANDERSON and OLIVER 1942).

Females homozygous for either of the three alleles in this report (other alleles may also be added) lack spermathecae which may be sperm storage spaces and also parovaria. However, ventral receptacles are present, and the lumen in the receptacle is normal and allows the storage of sperm. For some reason, though, the sperm in the receptacle cannot function over a long period of time. A probable relationship between the absence of the spermathecae and the lack of sperm motility has been studied by R. C. ANDERSON and is in preparation for publication.

Heterosis in compounds

The three compounds differed significantly in two of the three tests for viability, two of the three for fertility, and in one of the tests for fecundity. Although the order of magnitude for all three tests was lz^g/lz^s, lz^s/lz and lz^g/lz, the compounds lz^s/lz and lz^g/lz had no real difference in viability, and lz^g/lz^s and lz^s/lz did not differ significantly in fertility. Only lz^g/lz^s and lz^g/lz differed

in fecundity. The compounds were not so vigorous as control females, but they did show heterosis as compared to the homozygous alleles.

Compounds of the alleles were in general more viable, fertile, and fecund than the homozygous cultures of the alleles used in the compounds. Only two exceptions were observed in the tests. Compound lz^g/lz approximately equalled homozygous lz^g in viability and did not differ significantly from lz in fertility. In two other instances a compound did not show an increase in vigor greater than that measured for a homozygous allele not used in the compound. Compound lz^g/lz had the same degree of fertility as did homozygous lz^s, and compound lz^s/lz was not significantly higher in viability than lz^g. For all other tests, however, the compounds were more vigorous than the homozygous alleles and heterosis was observed. With respect to fecundity, the increase in vigor is expressed by fewer females falling into the low fecund groups and also by an increase in the range of number of offspring per culture. Stone (1942) also has reported increased fertility and fecundity in crossing strains of *Drosophila hydei*, with heterosis being observed in the F_1 of the crosses of geographic strains.

Interpretations of heterosis

Heterosis is observed as a general condition resulting from making compounds of lozenge alleles. Among the three alleles studied, in only two cases did an allele seem to dominate the other allele used in a compound. In lz^g/lz, the least vigorous of the three compounds, lz^g apparently dominated lz in the expression of viability, and lz tended to dominate lz^g as shown in the fertility test.

Although heterosis occurs as a result of the interaction between the alleles, no constant regularity in the interaction can be expected. In the tests, homozygous lz^g was the most viable of the three alleles, and lz^s was significantly the most fertile and fecund. In compounds, however, lz^g interacted with the least viable allele, lz^s, to produce the most viable compound, and compounds of lz^s and lz and of lz^g and lz had the same viability. The homozgyous allele which was the most fertile, lz^s, compounded with the least fertile, lz^g, to produce one of the most fertile compounds, although that compound did not differ significantly from lz^s/lz. In fecundity tests, lz^s, which was significantly the most fecund allele, did not increase the fecundity when in compound with lz any higher than did lz^g and lz when together. In homozygous condition, lz^g and lz did not differ in fecundity.

It seems probable that modifying genes which are present in the cultures of the homozygous alleles have a deleterious effect upon viability, fertility, and fecundity. Evidences which suggest this interpretation are found in comparisons of the vigor of the partially isogenic cultures with that of the original cultures. The type which as a result of crossing over had the two end portions of another X chromosome associated with lz^s showed an increase in viability, fertility, and fecundity. The new arrangement with lz^g did not increase viability, but it did show increased vigor as measured by fertility and fecundity. It will be recalled that the control females for the original tests had the left half of the

lz^{s}-bearing chromosome and the right half of the *lz*^{v}-bearing chromosome, and the control females tended to have a higher vigor than the lozenge alleles or their compounds. However, even though the results with the partially isogenic cultures suggest that modifying genes have an effect on vigor, heterosis was still observed in the compound of *v lz*^{v} *f* and *v lz*^{s} *f*. It was still true that the two alleles when brought together in the same female were significantly more viable, fertile, and fecund than either of the homozygous isogenic cultures. It seems, therefore, that interaction between the two alleles is expressed by increased vigor.

Heterosis may be the result of two general conditions. An individual may be more vigorous than either of the parental cultures used in the mating due to interactions among modifiers which are brought into the "hybrid," and the combinations of modifiers differ in the hybrid and parental cultures. It may also be true, at least with the lozenge locus in Drosophila, that two recessive alleles may interact in compounds, heterozygous for the two alleles, and show increased vigor in the manner suggested by EAST (1936).

Heterosis in the lozenge compounds is not due to any change in morphological characters which may be observed. The reproductive units which are lacking in the homozygous females (spermathecae and parovaria) are also absent in the compounds. Yet in the tests the compound females proved to be significantly more fertile and fecund than the homozygous alleles. All evidence points to the conclusion that the genes, lozenge alleles, control some physiological condition of the internal genitalia which inhibits the sperm in the homozygous cultures and to a lesser degree in the compounds. At least, the compounds have a smaller proportion of cultures which produce only one to three offspring as well as a wider range of fecundity than do the alleles in homozygous condition. Sperm inactivation in reproductive tracts of females has been reported in some of the speciation studies (PATTERSON 1942; PATTERSON, STONE, and GRIFFIN 1940, 1942) in which egg-hatch counts showed that a few eggs hatched in the first day of the test but that no eggs hatched subsequent to that time. In the egg-hatch counts with homozygous *lz*^{s}, only females were used in which copulation had been observed; and although some of the females produced eggs throughout the test, the number of offspring produced by any female tended to be small, and those offspring which hatched occurred only during the early part of the test.

In *Drosophila melanogaster*, genes exist which control the vigor of individuals, and mutations occur which cause other genes to act as modifiers in the development of vigor. Alleles of some genes, such as lozenge, affect the viability, fertility, and fecundity of individuals. However, the alleles of the locus do not necessarily act alike, and heterosis occurs as a result of compounds in which interaction between the recessive alleles occurs. The action of the genes on fertility and fecundity may in part be related to deficiencies in organs necessary for breeding, but results with compounds in the lozenge tests suggest that the genes may also control some physiological condition of the storage organs which affects the sperm stored there. There is a suggestion, therefore, that genes can

act as isolating factors by causing the development of a physiological condition detrimental to sperm viability in the ducts of the female. The preceding sentence does not imply that the lozenge-gene can or does act as an isolating factor. Homozygous lozenge females are very infertile when mated to any male. The data do indicate, however, that one of the actions of the lozenge-gene is related to loss of sperm motility in the genital ducts of the female. By analogy, therefore, one may postulate that genes can cause the development of physiological conditions which inhibit fertilization and, therefore, that isolating factors may be subject to gene control.

SUMMARY

Comparisons of the viability, fertility and fecundity were made for three recessive alleles of the lozenge locus (*lz*^{g}, *lz*^{s}, *lz*), their compounds and controls. All three homozygous alleles were associated with significantly lower viability, fertility and fecundity as compared with the controls.

Compound females were for the most part less vigorous in all three tests than were control females. However, compounding tended to produce heterosis in comparisons with homozygous females. Increased fecundity in compound females was associated with a lesser proportion of cultures in the low fecund classes and a wider range in number of progeny per fertile female.

Observations with partially isogenic cultures suggest that the regular stocks carried modifying genes which affected the vigor of the females. The data also indicate that heterosis occurs as a result of the interaction of the recessive lozenge alleles when they are brought into a compound.

No consistent relationship was found between the number of eggs and the number of progeny produced. Numerous eggs were found in sterile cultures and in those which produced only a few offspring. Among the *lz*^{g} as compared with controls, a larger proportion of the females which copulated proved to be sterile, but those which deposited eggs had an average daily production very close to that observed in control females, and many produced over most of the five-day period of the test. However, the average daily production of progeny by *lz*^{g} females was less than that of controls, and the offspring tended to appear in the early part of the test.

Morphological anomalies of the reproductive ducts were observed in the homozygous females, but the same deficiencies were seen in the more fertile and fecund compounds.

It is suggested that lower fecundity and possibly fertility in the homozygous females, and to a lesser extent in the compounds, is related to a decreased ability of the sperm to retain their power to fertilize the eggs and that the loss of viability of the sperm is due to genic control of some physiological condition of the reproductive ducts of the female.

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FREQUENCY OF DOMINANT LETHALS INDUCED BY RADIATION IN SPERMS OF DROSOPHILA MELANOGASTER

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THE proportion of the eggs laid by Drosophila females that fail to develop into adult flies is increased when the sperms used in fertilizing the females have been previously treated with ionizing radiation. It is generally believed that these induced failures of development are due to "dominant-lethal" changes in the genetic material of the treated sperms. These changes have been visualized alternatively as being closely related either to gene mutations or to chromosomal aberrations. Early investigators inclined toward the first view, but now the latter view is becoming firmly established. Most of the individuals carrying dominant lethals die in the egg stage, so that the proportion of eggs that fail to hatch is usually a good index of the frequency of dominant lethals. Determination of the proportion of organisms that attain, or fail to attain, some more advanced stage of development (such as pupation or emergence of adult fly) offers a better chance of scoring all the induced dominant lethals, but may more frequently be interfered with by external, accidental causes of death.

X-ray-induced dominant-lethal effects in Drosophila were first noticed by MULLER (1927). Quantitative experiments to investigate the general characteristics of the phenomenon were made soon afterwards by HANSON (1928) and then by TIMOFÉEFF-RESSOVSKY (1931). It was realized that the relationship between the dosage of radiation treatments and the frequency of dominant lethals is fundamental for the interpretation of the nature of these changes. GOWEN and GAY (1933) published graphs indicating that this relationship is of the type expected in the case of "single-event," or "single-hit," phenomena. Accordingly, these authors considered dominant lethals as special types of gene mutations. Their evidence is not adequate, however, for the solution of this problem. In the first place, the experiments did not yield details in the most critical range of the dosage-effect relationship—that is, at low dosage. In the second place, these authors did not measure the actual proportion of organisms developing in any single culture, but simply compared the total numbers of flies emerging from experimental and control cultures raised under parallel conditions. Finally, comparison of their results on recessive lethals with similar results by other authors suggests that their dosage measurements were in error by as much as a factor of 2.5 (SCHULTZ 1936). SONNENBLICK (1940) in a more recent experiment was mainly concerned with the direct observation of anomalies in the embryonic development accompanying dominant lethals. Within the sections of single eggs, he found distortions of various types in the mitotic

divisions, as well as normal figures. As a secondary objective, he determined the dosage-effect relationship for dominant lethals induced in sperms. This determination made it doubtful whether the relationship belongs to the one-event or to the many-event type.

Interest in investigating the nature of dominant lethals was stimulated by progress in the knowledge of X-ray effects on Drosophila sperm chromosomes (BAUER, DEMEREC, and KAUFMANN 1938) and of the nature of an increasing number of visible and recessive lethal mutations (DEMEREC 1937, 1939). Experiments on dominant lethals were begun in this laboratory in 1937. Preliminary results (DEMEREC, KAUFMANN, and HOOVER 1938) did not settle the question of the dosage-effect relationship at low dosage. A later effort directed to this end, however, was successful in establishing with some degree of reliability that the relationship belongs to the one-event type. The preliminary report of these findings (FANO and DEMEREC 1941) was soon followed by a reasonable interpretation of dominant lethals (PONTECORVO 1942), which will be discussed further in this paper.

In the course of our experiments we became interested in the variability of the frequency of dominant lethals depending upon the method of observation, the stock of flies used, and other causes of an accidental nature. Although the results of dominant lethal experiments are, on the whole, readily reproducible, the error in any particular determination of the frequency of dominant lethals is to a considerable and sometimes disturbing extent dependent on causes other than random sampling. Experiments in which eggs laid by individual females were observed separately showed that the percentage of failures of development depends on individual characteristics of the parent female, other conditions being equal. This effect becomes very apparent in control experiments with untreated sperms, where most of the observed failures of development occur among the offspring of certain parent females. Thus, in order to reduce the experimental error affecting the frequency of dominant lethals, it is necessary first to eliminate to some extent the causes of sterility in untreated material. One major factor causing such sterility was identified as a recessive gene (the *sst* or "semisterility" gene), which reduces by 50 percent or more the hatchability of the eggs of homozygous *sst* females (FANO 1942). Whenever *sst* is present in the stock under experiment, *sst* female parents contribute a highly variable percentage of the eggs of different collections from a mass culture. It follows that this one gene was probably responsible for certain striking variability effects; but its elimination did not solve the problem entirely, probably because of the presence of a large number of less striking analogous factors. Also, the variability effects exhibited by inbred laboratory stocks do not remain constant in time. Two different stocks which for a time exhibited a particularly low degree of variability lost this property in the course of one year. If variability is chiefly governed by genetic factors, it must be assumed that the frequency of those factors undergoes considerable change even in inbred stocks.

In view of this experience, an attempt was made later to select a stock

largely free of factors of the *sst* type, by establishing lines homozygous for a set of first, second, and third chromosomes derived from one female of proven high fertility. No success was obtained by this method.

The sex ratio of the offspring from X-rayed sperms is one particular aspect of the problem of dominant lethals that was studied from the very beginning. A shift in this ratio would indicate that sperms carrying the X chromosome and those carrying Y have different sensitivity to irradiation. HANSON (1928) reported a slight but not quite significant shift of the sex ratio in favor of males, while BARTH (1929) observed a significant shift in the same direction. GOWEN and GAY (1933) reported a significant shift, which increases regularly with increasing radiation dosage. Our own experiments were first quoted as not showing any significant shift (FANO 1941), but taken as a whole they are open to various interpretations.

We intend here to present a complete report on the experiments carried out in this laboratory.

MATERIAL AND METHODS

Various wild type inbred laboratory stocks were used in our experiments. Treated or control males were mated in mass to virgin females, and egg collection was begun after allowing a few hours (usually overnight) for mating. Most satisfactory results were obtained when both males and females had been kept with abundant food for a few days prior to mating. For a few days after mating, eggs were collected and counted on pieces of corn meal agar resting either on glass slides or on spoons. In order to score the number of hatched eggs, slides or spoons may be incubated without any further handling for the required time; but when the number of pupae or of adult flies is to be scored, slides or spoons must eventually be incubated in vials or bottles with additional food until the hatching of adult flies is completed. Later experience proved that for incubation it is more convenient to transfer batches of eggs of constant size (25) from spoons to slants of agar in vials. This procedure minimizes the chances of accidental death during development and permits a sufficiently accurate check of the number of eggs that have hatched. It also facilitates statistical comparison of the results obtained from different vials. This method was followed in the 1942 and 1943 experiments. If desired, eggs may be collected from single females.

X-ray treatments were carried out partly at Memorial Hospital, New York City, with the kind cooperation of DR. G. FAILLA and MR. L. D. MARINELLI, and partly in this laboratory. Neutron treatment was kindly carried out by the COLUMBIA UNIVERSITY cyclotron group, under the direction of DR. J. R. DUNNING.

RESULTS AND DISCUSSION

(a) *Dependence on the radiation dosage*

The results of four different series of experiments are plotted in figure 1. The ordinates show the percentage of eggs collected that developed to the stage of adult fly. The abscissae show the radiation dosage, expressed in roentgens

for X-ray experiments and in "neutron units" for neutron experiments, the two scales being arbitrarily superimposed on the basis of one neutron unit per every five roentgens (FANO 1944). Each point of the 1937, 1940, and 1942 series is based on the collection of about 1000, or more, eggs; each point of the 1943 series is based on about 600 eggs in figure 1a and on 2500 eggs in figure 1b.

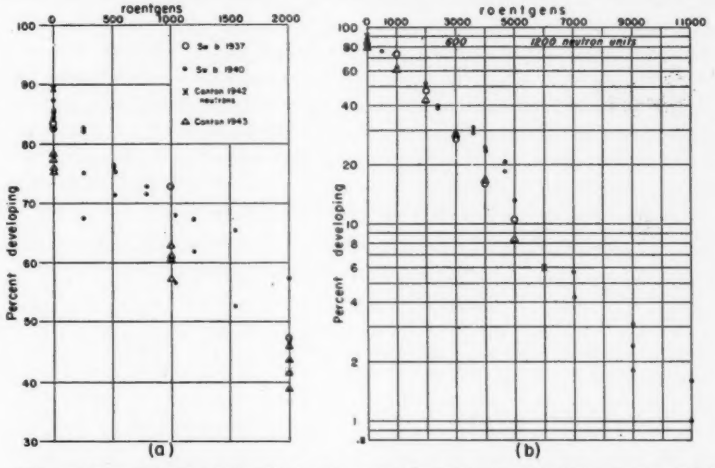

FIG. 1.—Dosage-effect relationship in the production of dominant lethals, showing detailed results of separate experiments. (a) Linear scale. Each point represents one experiment. (b) Semi-logarithmic scale. Points at low dosage represent averages of several experiments.

Three different intensities were used for the X-ray treatments of the 1940 series shown in figure 1; but no correlation between the intensity and the frequency of dominant lethals was apparent, so that no indication of the intensity is given in the figure.

The investigation of dominant lethals was undertaken mainly to determine whether or not the major part of the dominant-lethal effects may be attributed to chromosomal rearrangements of the same general type as those that are detected in individuals attaining advanced development. This hypothesis was suggested by the information available regarding viable chromosomal rearrangements (translocations, inversions, etc.), according to which related but inviable types of rearrangements (dicentrics, large deficiencies, etc.) should occur with considerable frequency. The results presented in this paper strongly discredit this hypothesis, especially because at low dosage the frequency of dominant lethals appears to be directly proportional to the dosage, and because the rate of proportionality is as high as 15–20 percent per 1000 roentgens. Since the publication of the preliminary report the significance of these findings has been repeatedly discussed by FANO (1941) and in particular by PONTECORVO (1942) who succeeded in formulating an acceptable interpretation for the origin of the majority of dominant lethals. He assumes that single chromo-

somal breaks are produced by radiation at a rate proportional to the radiation dosage and that those that do not undergo restitution nor participate with other breaks in a rearrangement undergo fusion of the sister chromatid broken ends. This starts a cycle of breakage-fusion-bridge such as the one described by McCLINTOCK (1941) in maize. Through this process, the orderly distribution of a balanced chromosomal complement in successive nuclear divisions is disturbed; this eventually results in genetic unbalance and death. The data presented in this section are satisfactorily explained by PONTECORVO's interpretation. The type of dominant lethals mentioned earlier (dicentrics, etc.) probably occurs with considerable frequency at higher dosages and may thus be responsible for the slight downward curvature shown by the semi-logarithmic plot of the percentage of eggs that developed into adult flies (fig. 1b).

(b) *Effect of dominant lethals on the sex ratio*

The ratio of the numbers of female and male adult flies that emerged in our experiments is shown below, together with its standard error:

	Control	1000r	2000r	3000r	4000r	5000r
Sw-b 1937	1.16±.04	1.12±.07	1.03±.08	1.03±.09	1.10±.14	.79±.18
Ore-R 1937	1.09±.04	.98±.06	1.16±.08	1.14±.11	1.04±.15	.92±.18
Canton 1943	1.04±.05	1.02±.05	1.02±.06	1.05±.07	1.07±.10	.99±.14

	Control	260r	520r	780r	1040r	1200r
	1.02±.03	1.08±.03	1.06±.03	.99±.04	1.08±.06	.99±.05

	1550r	2000r	2400r	3600r	4000r	4680r
Sw-b 1940	.98±.06	1.10±.06	1.00±.06	.99±.08	.93±.09	1.08±.10

5000r	7000r	9000r	11,000r
.89±.16	.97±.15	1.04±.17	.84±.18

The standard error is rather large, being approximately two divided by the square root of the number of flies examined. For instance, reduction of the error to .01 would require the examination of about 40,000 flies.

These results agree in a general way with previous reports by several authors (HANSON 1928; LAURINAT 1930; GOWEN and GAY 1933; etc.), indicating the existence of a slight excess of females in untreated material and a tendency toward an excess of males in the case of heavy treatments. The data reported by GOWEN and GAY are more definite than ours in indicating a shift of sex ratio. According to these authors, the sex ratio declines exponentially with increasing dosage, at a rate approximately 20 times lower than the rate of decline of the percentage of organisms developing. Such a low rate of decline is consistent with our results, but a much higher rate would not be.

The slightness of the effect of irradiation on the sex ratio, assuming that this effect is significant at all, requires special consideration. Cytological data on the distribution among chromosomes of breaks induced by radiation lead one to expect that approximately one out of every five breaks induced in female-determining sperms will occur in the X chromosome. As was pointed out earlier, PONTECORVO assumed that single breaks frequently produce a dominant-lethal effect through a breakage-fusion-bridge cycle of chromatids which causes

genetic unbalance. According to this assumption, breakage of the Y chromosome of male-determining sperms could not act as a dominant lethal because no genetic unbalance should result from fragmentation of Y in cells of the embryo. Therefore, only four dominant lethals would be expected in males for every five induced in females, and the sex ratio would decline at a rate four to five times lower than the total percentage of males and females developing. Such a high rate of decline would reduce the sex ratio by about 10 percent in the case of 2500-r treatments, in disagreement with all the experimental evidence. This evidence, on the contrary, indicates that either: (1) the X chromosome contributes much less than one-fifth of all dominant lethals observed at low dosage; or (2) the numbers of dominant lethals contributed by the X and Y chromosomes are roughly comparable.

We see no reason for supporting the first alternative. The second, however, is acceptable, provided that it is possible to modify PONTECORVO's theory by assuming that the breakage-fusion-bridge cycle of the genetically "neutral" Y chromosome may cause death otherwise than through genetic unbalance. Some grounds for this assumption are furnished, for example, by two concurrent lines of experimental evidence, for which we are indebted to DR. B. MCCLINTOCK (MCCLINTOCK 1938 and unpublished). (1) The chromatin bridges occurring during breakage-fusion-bridge cycles in maize do not necessarily break as soon as they are pulled by the spindle, but may persist long after anaphase. When this happens, and the nuclear division is followed by complete cell division, the bridge is eventually broken at the position of the new cell wall. However, the membrane forming around each of the nuclei extends to envelop the fragments of the broken bridge so as to reach the cell wall. (2) Cytological observations show that a nuclear membrane, whose shape is initially irregular because it envelops fragments removed from the bulk of the chromatin, has a strong tendency to become more nearly spherical so as to bring together all the chromatin into a single unit. All this suggests that when a bridge occurs in a division that is not followed by formation of a new cell wall, the bridge may persist and a single nuclear membrane may be formed that encloses all the chromosomes of the division figures and produces a dumbbell-shaped structure. The membrane might then presumably force the structure into a more nearly spherical shape, so that the ultimate effect of the bridge would be to induce polyploidy, whose degree might be still further increased by repetition of the same mechanism. In Drosophila, fertilization of the egg is followed by a series of nuclear division cycles without formation of separate cells.

It seems plausible then to assume that dominant lethals may be due to interruption of the breakage-fusion-bridge cycle by a persisting bridge. This interruption would not necessarily follow the first (radiation-induced) breakage, but might start from any breakage along a breakage-fusion-bridge cycle. The special genetic "inactivity" of the Y chromosome would then not come into play, and no difficulty would stand in the way of attributing to X and Y equal participation in the production of X-ray-induced dominant lethals. Dominant lethals induced according to this interpretation might be expected to cause

death early in the egg stage. Dominant lethals causing death in the larval stage, which occur with minor frequency, should then be attributed to some different mechanism, connected with less drastic dislocation of cell multiplication and perhaps more closely related to gene mutations.

(c) *Comparison of different criteria for scoring dominant lethals*

The 1937 experiments definitely showed that death during the pupal stage occurs independently of the radiation treatment with the low, practically con-

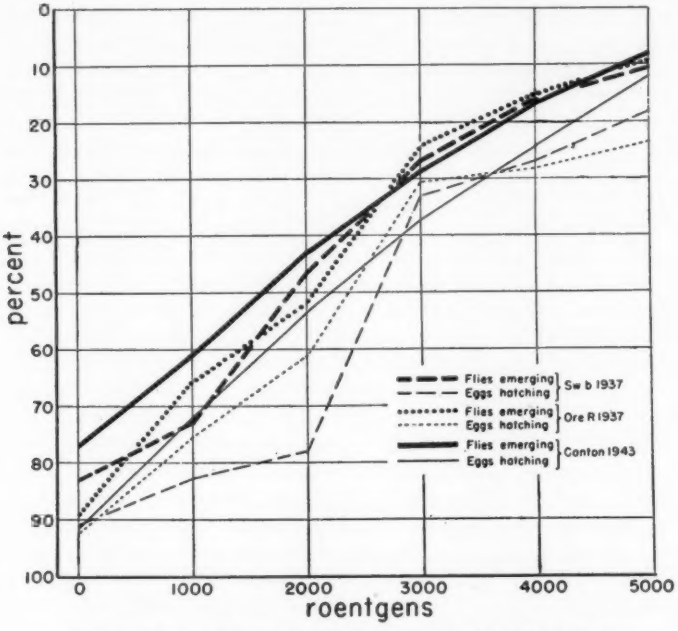

FIG. 2.—Dosage-effect relationships for various stocks of flies and for different methods of scoring dominant lethals.

stant, frequency of a fraction of 1 percent. The egg and larval stages then remain for further consideration, as those during which death occurs most frequently. Death in the egg stage is scored by measuring the frequency of egg-hatching, while death in egg or larval or pupal stage is scored through the frequency of emergence of adult flies. Three different sets of experiments, in which frequencies of both egg-hatching and fly-emergence were determined, gave the results plotted in figure 2, where the percentage of organisms developing to the stage of egg-hatching or fly-emergence is plotted against the X-ray dosage as in figure 1a. The two curves corresponding to each set of experiments are, on the whole, approximately parallel and not very far apart from one another. This means that the proportion of organisms that die during the larval

stage is comparatively low (about 10 to 20 percent) and approximately the same for different radiation treatments.

To evaluate this result, we may consider what should be expected under certain assumptions. (1) Dominant lethals produce death during the egg stage, while death during the larval stage is due to accidents independent of genetic effects. If so, the ratio

$$\frac{\text{percentage of organisms dying in larval (or pupal) stage}}{\text{percentage of all eggs hatching (regardless of outcome of later development)}}$$

ought to be independent of the treatment—that is, in figure 3 the ordinates of the two curves of each pair should be in a constant ratio, if measured from the level of "zero percent," and the curves themselves should converge with increasing dosage. (2) Dominant lethals produce death during the egg or larval stage; uncontrolled factors independent of the treatment determine in each case whether death occurs before or after the egg-hatching. If so, the ratio

$$\frac{\text{percentage of organisms dying in larval (or pupal) stage}}{\text{percentage of all failures of development}}$$

should be independent of the treatment—that is, in figure 2 the ordinates of the two curves of each pair should be in a constant ratio, if measured from the level of "one hundred percent," and the curves themselves should diverge with increasing dosage. The results shown in figure 2 are intermediate between those to be expected under these extreme hypotheses.

The 1000 r and 2000 r treatments of the 1937 Swedish-b series yielded one instance of appreciable departure from the customary result. The frequency of death in the egg stage was particularly low in these experiments, suggesting that some accidental condition peculiar to the experiments had prolonged the development of otherwise-doomed organisms beyond the time of egg-hatching. Because of this result, more reliance was put in later experiments on fly-emergence than on egg-hatching as a criterion for determining dominant lethals; but no other similar instance occurred subsequently.

(d) *Comparison of results obtained with different stocks*

Results of the 1937 experiments with Oregon-R and Swedish-b stock and of the 1943 experiments with Canton are shown in figure 2. The treatments of Oregon-R and Swedish-b were simultaneous.

It may be seen from figure 2 that more dominant lethals (as expressed by the differences between the "percent developing" in treated and in untreated material) have been observed for the Oregon-R than for the Swedish-b stock at all dosages. This result was interpreted in a preliminary report (DEMEREC et al. 1938) as an indication of a systematic difference of sensitivity between the two stocks. The significance of this indication has become doubtful in view of the more recent experience on the error involved in each experiment.

(e) *Reproducibility of experiments after long time-intervals*

Comparison of the data of the 1937 and the 1940 experiments with Swedish-b

stock, shown in figure 1, indicates that the results of dominant-lethal experiments remain constant over a period of time, within the limits of variability discussed in the following section. Results of the 1942 and the 1943 experiments with untreated Canton stock, shown in figure 1a and in figure 3, indicate that the frequency of failures of development in untreated material in that stock had undergone a significant, though not great, change in the course of one year.

(f) *Variability between the results of repeated experiments*

The data in figure 1 show a considerable degree of variability, even though the amount of material used in determining each point was rather large.

The first step in the statistical analysis of this variability was to test the homogeneity of the results—that is, of the "percent developing"—obtained from all the separate batches of eggs collected throughout each single experiment. These homogeneity tests were carried out by the χ^2 method for the 33 experiments of the 1940 series in which the radiation dosage was less than 5000 r. The degree of homogeneity is characterized, as usual, by a "probability level," and each experiment may be classified according to its probability level. Classification of the 33 experiments gave the following distribution:

PROBABILITY LEVEL				NUMBER OF EXPERIMENTS
between	1	and	.5	1
"	.5	"	.1	4
"	.1	"	.05	2
"	.05	"	.01	14
"	.01	"	.001	6
"	.001	"	.0001	2
"	.0001	"	.00001	1
"	.00001	"	.000001	2
"	.000001	"	0	1

The prevalence of low levels of probability in this table indicates a variability considerably greater than may be attributed to random sampling. This finding led to the investigations of variability summarized in the introduction.

The next step in the analysis was to determine whether or not the differences between the results of repeated experiments at the same dosage are significant. To this end, an analysis of variance was carried out for the results obtained from all the separate batches of eggs collected in the course of all the experiments at the same dosage. The 33 experiments were arranged in 12 groups according to dosage, and the variance "between experiments" was compared with the variance "within experiments" for each group. (The particular values of the numbers of degrees of freedom encountered made it possible in every case to reduce the z test either to a χ^2 or to a t test.) Eight of the 12 groups did not show any significant excess of variance between experiments. The group of four experiments at 260 r yielded the very significant value $\chi^2 = 79.5$ for the three degrees of freedom between experiments. The pair of experiments at 1550 r yielded $t = 2.77$, a little below the .01 level of significance; the pairs at 1036 r and 2000 r yielded $t = 4.87$ and $t = 4.12$, respectively, very much below that level.

Since the Canton stock showed a low degree of variability in the 1942 neutron experiment, it was used again in the 1943 X-ray experiments, which were especially designed for a study of variability. Four closely comparable sets of experiments were run in successive weeks. The number of eggs hatching and of flies emerging in each of the vials to which 25 eggs had been transferred was recorded, and χ^2 homogeneity tests were carried out for the counts of all vials within each single experiment. Twenty-four experiments (at six different dosages) were classified according to the homogeneity of the egg counts, and again according to the homogeneity of the fly counts. The results of these classifications were:

PROBABILITY LEVEL	CLASSIFICATION BY EGG COUNTS (NO. OF EXPERIMENTS)	CLASSIFICATION BY FLY COUNTS (NO. OF EXPERIMENTS)
between 1 and .5	6	8
" .5 " .1	15	12
" .1 " .05	1	2
" .05 " .01	2	2

These results show, on the whole, only a slight trend toward non-homogeneity. Chi-square homogeneity tests were then carried out, grouping together the counts of all vials (100 in number) at each dosage, even though they belonged to four different experiments. The following probability levels were obtained:

DOSAGE IN r	PROBABILITY LEVEL (EGG COUNTS)	PROBABILITY LEVEL (FLY COUNTS)
0	.0001	.04
1000	.30	.60
2000	.78	.34
3000	.39	.30
4000	.22	.01
5000	.20	.67

The non-homogeneity appearing here is not very great. However, it is possible that the contributions to the χ^2 arising from the three degrees of freedom "between the experiments" at each dosage are actually significant, but that their significance is obscured because they are added with the contributions arising from the 96 degrees of freedom "within the experiments." A test of analysis of variance was therefore carried out, comparing for each dosage the variance between and within experiments. Because of the large number of degrees of freedom within experiments, the z tests reduced to χ^2 tests. The probability levels for the variance between experiments are:

DOSAGE IN r	PROBABILITY LEVEL (EGG COUNTS)	PROBABILITY LEVEL (FLY COUNTS)
0	.01	.7
1000	.2	.3
2000	.0004	.04
3000	.04	.5
4000	.0003	.0001
5000	.1	.1

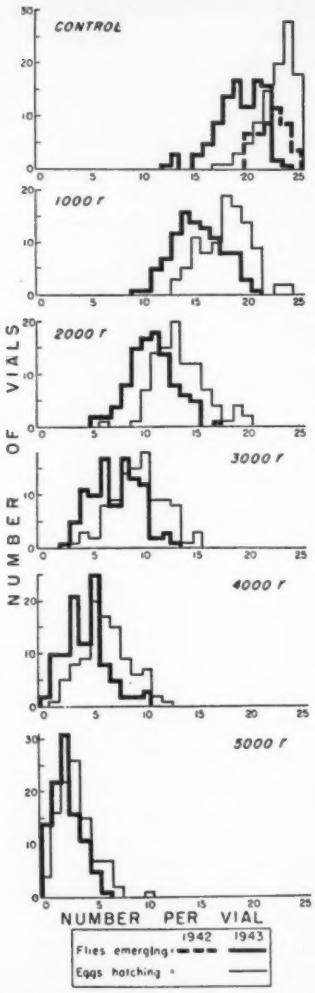

FIG. 3.—Numbers of eggs that hatched and of flies that emerged in vials where 25 eggs of Canton stock had been incubated (see text).

The variability shown by these results, even though not very great, is considerable and is roughly comparable to that exhibited by the analysis of variance of the 1940 experiments, since in both cases the variability between experiments is significant in one-third of the tests. As suspected, a χ^2 test of homogeneity does not necessarily yield a satisfactory indication of the vari-

ability between experiments; for instance, the egg counts at 2000 r and 4000 r do not exhibit any significant lack of homogeneity in the χ^2 test, whereas the analysis of variance brought out a very significant excess of variance between the experiments at those dosages.

The complete results of the 1943 experiments with Canton stock—namely, the numbers of eggs that hatched and of flies that emerged from each of the vials where 25 eggs had been incubated—are shown in figure 3 in the form of histograms. Each histogram presents results obtained in four separate experiments with the same treatment, involving a total of 100 vials. The variability exhibited by each histogram is mainly due to random sampling, as indicated by the χ^2 homogeneity tests. The shift of the histograms to the left with increasing dosage is the expression of the dosage-effect relationship. The heavy-lined and the thin-lined histograms illustrate the comparative results of different methods of scoring dominant lethals (see subsection (c) and fig. 2). The broken-lined histogram in the controls shows the results of an experiment performed with the same stock in 1942.

These studies of variability lead to the conclusion that, under favorable conditions, the uncertainty involved in a determination of dominant lethals may be only slightly greater than the unavoidable sampling error. At times, however, the error may be considerably greater than this, as shown by the results of the 1940 experiments—whose variability should not be attributed entirely to defective technique—and by the subsequent discovery of *sst* and of similar genetic factors causing variability. The observation that the χ^2 test sometimes fails to detect significant differences between the results of separate experiments may account for the discrepancy between our finding of considerable variability and DEMPSTER's (1941) report that results of his unpublished experiments were homogeneous on the basis of χ^2 tests.

SUMMARY

The dependence of the frequency of dominant lethals on the radiation dosage agrees with PONTECORVO's interpretation of the origin of dominant lethals. Dependence of the sex ratio on the dosage, however, requires some modification of this interpretation. The proportion of organisms that die during the larval stage is approximately the same for different radiation treatments of the parent males. Equal treatments induce approximately the same frequency of dominant lethals in different stocks of flies. The results obtained with the same inbred stock at different times are qualitatively reproducible, but quantitative differences are noticeable. The uncertainty involved in the determinations of the frequency of dominant lethals may be considerably greater than expected on the basis of the sampling error alone.

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EXPERIMENTS ON MUTATIONS INDUCED BY NEUTRONS IN DROSOPHILA MELANOGASTER SPERMS

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TWO somewhat puzzling phenomena have been reported in the literature on neutron-induced lethals in Drosophila. NAGAI and LOCHER (1938), in the first work on this subject, kept the offspring of different neutron-treated males separate during the tests for sex-linked lethals and reported that the lethals were not distributed at random among the offspring of different males. Two or more lethals occurred within the offspring of a single male with a frequency that was considered significantly in excess of its expected value. This was attributed to a hypothetical "grouping" of lethals within the chromosomes of adjacent sperms. Although the interpretation of this effect was not quite satisfactory, the report did not give rise to any related investigation, until NISHINA and MORIWAKI (1941) attempted to detect grouping of lethals within single chromosomes. These authors proceeded on the assumption that if neutrons are especially likely to produce an accumulation of lethals within the X chromosomes of adjacent sperms, they should also frequently produce more than one lethal within any single chromosome. The detection of a striking effect of grouping was reported from this experiment.

In view of the considerable importance of these findings for the interpretation of radiation effects, and since the technical methods of NISHINA and MORIWAKI were open to some criticism, it appeared worth while to repeat the basic experiments on grouping effects, using modified methods.

Many of the X-ray-induced sex-linked recessive lethals detected by the standard *ClB* method are known to be associated with chromosomal rearrangements affecting the X chromosome. The usual interpretation of this association attributes such lethals to a secondary ("position") effect of the rearrangements. Attempts to account for all the observed dose-effect relationships on the basis of this interpretation, however, have not been quite successful (*cf.* FANO 1941). Therefore, it also appeared worth while to obtain some data on the association of lethals and chromosomal rearrangements in neutron-treated material.

Finally, it seemed advisable to gather some new data on the efficiency of neutrons in producing dominant lethals, even though some information on this subject had already been published by DEMPSTER (1941).

MATERIAL AND METHODS

An inbred wild type Canton stock of *Drosophila melanogaster* was used in this experiment. Young males were neutron-rayed with the COLUMBIA UNIVERSITY

[1] Cytological analysis was done principally by DR. E. SUTTON, now at THE JOHNS HOPKINS UNIVERSITY, Baltimore, Maryland. It is a pleasure to acknowledge her substantial contribution.

cyclotron, which was kindly made available and operated for us by the cyclotron group, under the direction of DR. J. R. DUNNING.

The instrument measuring the neutron intensity was calibrated with a Victoreen dosimeter, using the 25 r chamber. Because of this circumstance, the "neutron unit" corresponding to the reading of 1 r on our dosimeter may be slightly greater than the conventional neutron unit determined using the 100 r chamber. In any case, the conversion of any one of these units into units of energy delivered to a unit volume of tissue is not very accurate. It is usually estimated (AEBERSOLD and LAWRENCE 1942) that one neutron unit is energetically equivalent to a value between 2 and 2.5 roentgens of X-rays.

Two groups of males were treated with about 600 and 1200 neutron units, respectively, and bred as follows:

(1) 236 males treated with 1200 units were mated separately, each to four or five *ClB/ec ct v g* virgin females. The F_1 Bar females of each successful culture were mated singly with *ec ct v g* males. F_2 maleless cultures containing at least 25 females were classified as carriers of lethals. F_2 wild type females of each of these lethal cultures were further mated with *ec ct v g* males. The F_3 generation thus obtained was subjected to genetic and cytological tests:

(a) Analysis of the crossover classes of the F_3 served to determine the approximate location of the lethal and its possible association with a chromosomal rearrangement. This method was not entirely satisfactory, as shown below, because of the lack of markers to the right of *g* in the stock employed. (Use of genetic tests with a more complete set of markers had previously met with difficulties on account of low viability.) (b) Cytological analysis was made of the salivary gland chromosomes of at least five non-*v* female larvae from each F_3 culture in order to detect any rearrangements affecting the X chromosome. Although particular attention was devoted to a search for deficiencies of considerable size, no attempt was made to detect deficiencies involving only a few bands.

Tests (a) and (b) were carried one generation further, when necessary for their completion.

(2) The other males that had been treated with 1200 units were mated in mass to Canton virgin females. Eggs from this mating were collected on spoons with corn meal agar for a few days, transferred in batches of 25 onto corn meal-agar slants in vials, and incubated at 25°C. The numbers of eggs hatched and of adult flies emerging in each vial were scored.

(3) Males treated with 600 units were bred as in (2).

(4) Five males treated with 600 units and previously used in experiment (3) were later mated singly with virgin *ClB/ec ct v g* females (but separated on the 12th day after treatment), and their offspring were tested as in (1).

One control experiment on dominant lethals, using untreated Canton males, was carried out with the technique indicated in (2).

RESULTS

Experiment (1) gave the following results:

Males yielding successful *CIB* tests		170	
X chromosomes tested		998	
X chromosomes tested per male:	minimum		1
	maximum		17
	average		5.9
X chromosomes acting as lethal carriers		60	
X chromosomes carrying one lethal without rearrangement			32
X chromosomes from which two different lethals without rearrangements were separated			2
X chromosomes carrying one lethal associated with a rearrangement			24
X chromosomes carrying independent lethal and rearrangement			2
Total number of lethals separated from one another		62	
Total number of lethals separated from one another and from rearrangements			38
Number of lethals associated with rearrangements			24

One lethal-carrying chromosome was lost before analysis and was arbitrarily classified as carrying one lethal without rearrangement. The test of one other chromosome yielded only *v g* males (with one exception, which was *v* alone), although crossing over in the *v-g* region appeared normal, judging from the F_2 females. A plausible interpretation is that there were two different lethals in the *v-g* region; but for practical purposes this chromosome, too, was arbitrarily classified as carrying one lethal without rearrangement.

The observed distribution of lethals among the offspring of different males must be compared with the distribution to be expected according to the hypothesis that lethals are distributed at random (that is, without exhibiting any grouping effect). The "expected" distribution would be a Poisson series, if the same number of tests were carried out in the offspring of each male. This condition is not fulfilled, but the deviation from it can be taken into account in computing a "corrected" expectation. The observed and expected distributions are:

Lethals per male	0	1	2	3	4	5
Observed	125	33	10	1	1	0
Expected: (Poisson)	119.5	42.1	7.4	.9	.1	.0
(Corrected)	120.7	40.1	7.8	1.2	.2	.0

The observed frequency of occurrence of two different lethals in the same X chromosome must be compared with the frequency to be expected according to the hypothesis that lethals are distributed at random among all treated X chromosomes (that is, without exhibiting any grouping effect). The apparently obvious basis for calculating the expected frequency is to assume that all 62 different lethals were distributed at random among the 998 chromosomes tested. The expected frequency of chromosomes with 0, 1, 2, etc., lethals would then constitute a Poisson series. It may be argued, however, that the coincidence of two lethals in the same chromosome may escape detection when one lethal is associated with a chromosomal aberration. This argument may be taken into account in the calculation by assuming that only the 38 different lethals that are not associated with rearrangements are distributed at random. However, the values obtained using either basis of calculation do not differ

greatly from each other or from the observed value, as is shown by the following table.

	FREQUENCY OF OCCURRENCE OF TWO LETHALS IN THE SAME CHROMOSOME
Observed	2
Expected: All lethals at random	1.8
Only lethals without rearrangement at random	.7

The expected frequency of occurrence of three lethals in the same chromosome is less than .05, and no such case was observed.

Experiment (4) yielded three lethals out of 156 chromosomes tested. Two of these lethals were without rearrangement, the other was associated with the intercalary translocation of a section of X to 3L.

A few other findings incidental to the cytogenetical analysis will be reported here, even though they involve small numbers of observations and do not appear to disagree with other information relating to similar questions. Three lethals (all of which were associated with rearrangements) showed phenotypical effects—namely, one Notch and two echinus. Among all the 25 observed associations of lethals and rearrangements, no deficiency was detected. At least six of these rearrangements involved more than two breaks. In five cases, two breaks were observed within one numbered "division" of the X chromosome or within two adjacent "number sections." Among the 19 cases where the lethal appeared to be associated with a simple inversion or reciprocal translocation, 12 involved an inversion and seven a translocation. Since the numbers of X-ray-induced reciprocal translocations and of inversions affecting any given chromosome limb are known to be about equal, and since inversions involve a double chance of producing a lethal (one at each end point), the observed ratio of 12 to seven is not unexpected. Finally, nine cases of cytologically detected rearrangements affecting the proximal region beyond *g* were not detected by the genetic test.

The dominant lethal experiments yielded the following percentages of eggs undergoing full development:

	Controls	600 r	1200 r
Percentage	89.6	27.2	6.0

Parallel experiments on X-ray-induced dominant lethals, carried out a year later with the same stock and the same technique gave the following results:

	Controls	1000 r	2000 r	3000 r	4000 r	5000 r
Percentage	76.9	60.8	42.6	28.5	16.5	8.3

Further details on these results (including graphs) are given in a separate paper on dominant lethals (DEMEREC and FANO 1944).

DISCUSSION

The interpretation of "grouping effects" is based on the lack of uniformity of the ionization produced by neutrons, which is concentrated along compara-

tively few and narrow paths of recoil protons. Because of this lack of uniformity, any region of tissue so small that it stands only a slight chance of being traversed by even one proton during the entire treatment will be particularly strongly affected if it does happen to be so traversed. Hence the neutron effects are expected to be "grouped" within regions of such size. In the usual treatments of Drosophila sperms, equivalent in order of magnitude to a few thousand roentgens of X-rays, each region whose diameter is of the order of magnitude of 1 μ has about an even chance of being traversed by a neutron. Therefore, grouping effects should be expected only when the region taken as a unit in connection with the grouping effect is smaller than 1 μ.

The grouping reported by NAGAI and LOCHER refers to a cluster of adjacent ripe sperms of a male, occupying a region very much larger than 1 μ, and therefore is unexpected from the physical standpoint. The results reported in the present paper, on the contrary, agree with the physical considerations, inasmuch as they do not show any significant grouping of that type, even though the number of cases of "one lethal per male" is somewhat smaller than expected, both in our experiment and in that of NAGAI and LOCHER. Actually, their conclusion about the existence of "grouping" seems to derive from a comparison of the results obtained with neutrons and γ-rays; but it is not supported by any calculation of the probable effect of random distribution of lethals to be expected in each case. Inspection of the tables published by NAGAI and LOCHER shows that the average number of lethals per male (and hence the chance of random coincidence) was much smaller in their γ-ray series (19/89) than in the neutron series (44/69). A copy of the original records has been made available to us through the courtesy of MISS NAGAI and DR. ALTENBURG. The information supplied by these records does not permit us to compute the "exact" but only the Poisson expectation, which compares with the observed results as follows:

Lethals per male	0	1	2	3	4
Observed	40	16	11	2	0
Expected (Poisson)	36.6	23.3	7.4	1.6	0.2

As in our results, the class with one lethal per male shows here some deficiency, compensated by excess in the classes with no and two lethals (while no excess is found in the class with three lethals). The discrepancy ought to be further reduced if the "exact" expectation were calculated; but it does not appear to be significant even as it is. In the γ-ray series by NAGAI and LOCHER, about two cases with two lethals per male should have been expected. The fact that none was found can be interpreted as a sampling error.

The "grouping" of lethals in single chromosomes reported by NISHINA and MORIWAKI appears at first to be fairly plausible, in view of the size of a chromosome and of the data on the physical action of neutrons considered earlier. However, owing to the elongated shape of chromosomes, it might not be very likely for a neutron hitting one point of a chromosome to hit the same chromosome at another point far removed from the first. NISHINA and MORIWAKI themselves pointed out that their method cannot distinguish the existence of

two different lethals unless they are separated by a considerable distance along a chromosome. Our data, presented in this paper, do not show any significant grouping of the kind reported by NISHINA and MORIWAKI and appear definitely to exclude the possibility that such grouping would occur with the high frequency (over 40 percent) reported by them. To explain the discrepancy between the two experiments, it may be noted that two different lethals were actually isolated in only one out of the eight cases of grouping reported by NISHINA and MORIWAKI. In two other cases, the grouping involved one lethal and one phenotypical (*ct*) change, but no evidence was offered indicating that the two changes were distinct. In the remaining five cases the existence of two independent lethals was argued on the basis of deviations from the expected sex ratio in the F_2. In view of the presence of numerous genetic markers which may reduce the viability of hemizygous males, this criterion does not seem quite reliable. On the one hand, no control data were shown supporting the validity of the method; on the other hand, in our experiments a sex ratio exceeding 3:1 in the F_2 appeared commonly in cases where the crossover classes indicated the existence of only one lethal.

The value found by us for the proportion of lethals associated with chromosomal rearrangements (about 40 percent) is one of the highest on record. Comparable information on X-ray-induced lethals may be derived from OLIVER's (1932) data, which indicate that the corresponding proportion is about 10 percent for a treatment producing about 6 percent lethals. This result may be in error by defect, since it is based on a genetic method using only the three markers *sc*, *v*, and *f*. DEMEREC (1937) has obtained values ranging up to 40 percent with X-ray treatments roughly equivalent to our neutron treatment. It may be argued, however, that DEMEREC's sample of lethals was selected by means of phenotypical effects, which may themselves be particularly closely associated with rearrangements. At any rate, the value obtained with neutrons is not surprisingly high in view of the evidence indicating that, as a rule, neutron treatments produce fewer gene mutations and more (or at least not fewer) chromosomal rearrangements than energetically equal doses of X-rays. In fact, the rate of production by neutrons of sex-linked lethals as a whole (that is, including the fraction associated with rearrangements) is about 0.7 times the comparable rate of production by X-rays. This result has been obtained by several authors since ZIMMER and TIMOFÉEFF-RESSOVSKY (1938) and is confirmed by the data of the present paper. Assuming that one neutron unit is equivalent to 2.5 r, 1200 units are equivalent to 3000 r; but 3000 r X-rays produce about 9 percent lethals in most wild type stocks, instead of the 6 percent found by us. In order to compare the efficiency of X-rays and neutrons in producing lethal gene mutations only, one must subtract from 6 percent the contribution of lethal rearrangements, as pointed out by GILES (1943). Thus 1200 units produce only about 3 to 4 percent (that is, about 60 percent of 6 percent) lethal gene mutations, while 3000 r X-rays produce about 6 to 8 percent of them. The neutron efficiency factor for gene mutations is then about 0.5 instead of 0.7. In the production of chromosomal rearrange-

ments, neutrons have appeared to be more effective than X-rays in all experiments reported (AEBERSOLD and LAWRENCE 1942; GRAY et al. 1940), except in the one on salivary-gland analysis of changes induced in Drosophila sperms (KAUFMANN 1941), where the effectiveness was about equal.

Even though the high value obtained for the proportion of lethals associated with rearrangements was not unexpected, its actual demonstration increases the difficulty of an already paradoxical situation (FANO 1941). Sex-linked lethals, as detected by the *ClB* method, consist of two components—namely, gene mutations (including "minute" rearrangements) and "gross" chromosomal rearrangements. The first component is generally supposed to be induced at a rate proportional to the radiation dosage, the second at a rate approximately proportional to the square of the dosage. How, then, can the sum of the two components be actually proportional to the radiation dosage, as observed, especially when the quantitative import of the second component is not negligible? Modified interpretations of the nature of the lethals associated with rearrangements may obviate this paradox, but they seem to meet with other serious difficulties. In the author's opinion, the paradox is but one aspect of some basic deficiency in our understanding of the complex relationships between the various types of genetic change and of their dependence on radiation dosage.

Earlier cytological investigation of neutron-induced chromosomal rearrangements in Drosophila (KAUFMANN 1941) has revealed a number of deficiencies of considerable size well in excess of the number of similar deficiencies present in comparable samples of X-ray-induced rearrangements. This led to the suggestion that neutrons can produce "one-event" deficiencies of larger size than are usually produced by X-rays (the upper limits for "one-event" X-ray-induced deficiencies being about 15 bands, according to DEMEREC and FANO 1941). The present cytological investigation of neutron-induced sex-linked lethals does not support this hypothesis. It must be noted, however, that none of the 12 neutron-induced deficiencies reported by KAUFMANN affects the X chromosome, most of them affecting 3R.

The dominant-lethal results fall in line with analogous results of X-ray experiments if one neutron unit is taken to be equivalent to about 5 r X-rays. This is best shown in figure 1b of the paper on dominant lethals by DEMEREC and FANO (1944). Since one neutron unit is generally recognized to be energetically equivalent to 2 or 2.5 r, it follows that neutrons are about twice as efficient as X-rays in producing dominant lethals. This result compares with DEMPSTER'S (1941) report setting the value of this efficiency factor at 1.5 The discrepancy between the two results is not very large, and its nature is not clear to the writer. Since most of the dominant lethals are now generally believed to be caused by single chromosomal breaks, the results reported in this paper indicate that neutrons are definitely more efficient than X-rays in producing chromosomal breaks in Drosophila sperms. This conclusion, however, does not agree with the conclusions drawn from the direct determination of the frequency of neutron-induced chromosomal rearrangements in the same ma-

terial: KAUFMANN's (1941) results set the efficiency ratio of neutrons and X-rays at about one, and DEMPSTER's (1941) at about 1.25. One possible interpretation of the disagreement is offered by DEMEREC's suggestion that an important percentage of neutron-induced dominant lethals consists of "one-event" deficiencies involving a considerable segment of chromosome (that is, one numbered "division" or more). To adjust this interpretation to the available evidence, specifically to that offered by KAUFMANN (1941), one should also assume that there is no important number of these deficiencies short enough to act as recessive instead of dominant lethals in the X chromosome, but that some of them may act as recessives in the autosomes, particularly in 3R. It is realized that the evidence available at present, including that supplied by the dominant-lethal experiment reported here, is not adequate to supply a clear picture of the comparative efficiency of neutrons and X-rays in producing the various types of chromosomal changes in Drosophila sperms.

SUMMARY

New experiments on the phenomena of "grouping" of sex-linked lethals reported by NAGAI and LOCHER (1938) and by NISHINA and MORIWAKI (1941) seem to disprove their existence. The theoretical difficulties raised by the interpretation of those phenomena are thus removed. A large percentage of neutron-induced lethals is associated with chromosomal rearrangements; certain difficulties that had previously been met in discussing this association are thus considerably increased. A new measurement of the frequency of neutron-induced dominant lethals is reported, but the comparative efficiency of neutrons and X-rays in producing various types of chromosomal change in Drosophila sperms is not satisfactorily elucidated thereby.

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COLLAPSE OF THE SEED FOLLOWING THE MATING OF HORDEUM JUBATUM×SECALE CEREALE

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INTRODUCTION

THE impairment in seed development often associated with wide crossing is readily observable in cereal species hybrids because of the persistence of the endosperm as a storage tissue and the large size of the normal caryopsis. Cytogeneticists interested in the cereals were early impressed, accordingly, with the importance of this stage in the life cycle of the plant for the general problem of hybrid incompatibility. It is not surprising to find, therefore, that a larger amount of work has been directed toward discovering the basis of hybrid seed failure in these plants than in any other group of angiosperms.

Some of the histological findings on the Gramineae as put forward in their original form, however, are seemingly at variance with more recent evidence on seed collapse as manifested in certain Dicotyledons. Additional data are desirable, therefore, on the question whether seed failure following hybridization of distantly related forms follows a common basic pattern or differs significantly from group to group.

MATERIALS AND METHODS

The present study of seed failure in the *Hordeum jubatum* (squirrel-tail barley) ♀×*Secale cereale* (rye) ♂ mating was planned along the lines of our earlier investigations of the crosses *Nicotiana rustica*×*N. glutinosa* (COOPER and BRINK 1940) and *N. rustica*×*N. tabacum* (BRINK and COOPER 1941). The histological methods employed are described in these earlier papers. Locally occurring plants of *H. jubatum* (n=14) were employed. The rye (n=7) used as the male parent in the intergeneric cross was of the Imperial variety as cultivated commercially in Wisconsin.

H. jubatum and *S. cereale* cross readily when the former is used as the pistillate parent, as QUINCKE (1940) has reported; and the hybrid seeds promptly begin to enlarge. Development continues for varying periods of time. Some seeds break down as early as four days; none has been found alive beyond 13 days. No seeds reach a germinable condition. The cross, therefore, is completely abortive. On the other hand, the normally autogamous *H. jubatum*, used as the control in these experiments, sets an approximately full complement of plump, viable seeds on artificial self-pollination. Development of the *H. jubatum*×*S. cereale* and *H. jubatum*, selfed, seeds was followed histologically from fertilization to the time of collapse of the hybrid.

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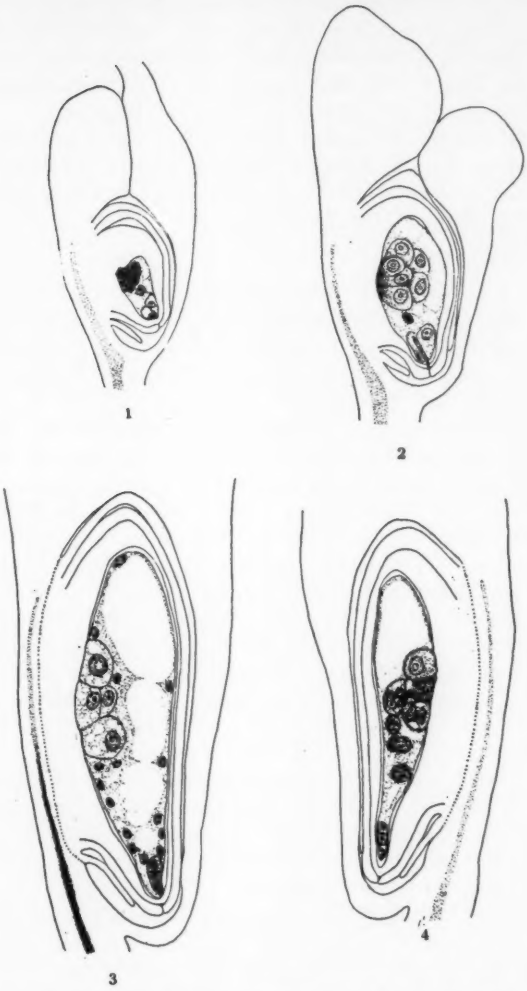

FIG. 1.—*Hordeum jubatum*. Longitudinal section through an ovary showing a mature megagametophyte. ×32.

FIG. 2.—*H. jubatum*. Longitudinal section through an ovary four hours after pollination. Fertilization has taken place and the primary endosperm nucleus is in a stage of mitosis. ×32.

FIG. 3.—*H. jubatum*. Longitudinal section of a seed at 28 hours. The nuclei of the endosperm are scattered in the peripheral layer of dense cytoplasm. Large vacuoles occupy the central region of the endosperm. The antipodal cells are large and highly vacuolate. ×32.

FIG. 4.—*H. jubatum*×*Secale cereale*. Longitudinal section of a seed at 28 hours. Four giant endosperm nuclei are located in the dense cytoplasm between the antipodals and the embryo. The cytoplasm of the antipodal cells is dense and many cells are binucleate. ×32.

The findings on the *H. jubatum*×*S. cereale* cross are in agreement with those of earlier investigators in showing that mitotic behavior of the endosperm in cereal hybrids may be markedly abnormal. This condition has not been observed in any Dicotyledonous mating thus far reported; and the fact, at first sight, may seem to preclude the possibility of bringing the known cases of seed failure under one point of view. Evidence is forthcoming from the present study, however, that the seeming non-conformity of endosperm behavior in cereal hybrid seeds rests upon a special relation of the antipodals to early development. This aspect of the problem will be discussed in detail in a following paper.

ENDOSPERM DEVELOPMENT IN H. JUBATUM

Fertilization usually occurs within four hours after self-pollination in *H. jubatum* and is delayed little, if any, in the cross with *S. cereale*. The frequency with which fertilization takes place following hand-pollination of castrated flowers is high in both matings.

Development of the *H. jubatum* endosperm proceeds with great rapidity following fertilization. Stages in division of the primary nucleus are often found in four-hour ovules (fig. 2). Occasionally the endosperm is already two-nucleate at this time. Further growth continues with a high degree of regularity by synchronous free nuclear division up to about 32 hours, at which time cell division is initiated; and at 48 hours a layer of cells completely surrounds the large central lumen (fig. 5). The increase in number of free endosperm nuclei proceeds geometrically as shown by the data in table 1 on modal number of nuclei at successive times during this interval. The modal number of endosperm nuclei at eight and at ten hours is four. The value has increased to 32 at 24 hours, the next period at which observations on selfed *H. jubatum* seeds were made. Further increases to 64 nuclei at 28 hours and to 128 nuclei at 32 hours are found. A few seeds were observed at 32 hours with a 256-nucleate endosperm. Cell formation is initiated in that portion of the 128-nucleate endosperm adjacent to the embryo and gradually progresses toward the antipodal region. The endosperm is almost completely cellular at the 256-nucleate stage, a few free nuclei remaining in the thicker layer of dense cytoplasm immediately adjacent to the antipodals.

The volume of the initial endosperm cell increases approximately six-fold prior to the division of the primary endosperm nucleus. This increase occurs in all dimensions but is greatest at the chalazal end where this cell expands beyond the antipodals altering the position of the latter from terminal (fig. 1) to median lateral (fig. 2). The increase in volume of the endosperm during the period of free-nuclear division is likewise great. Some of the nuclei become distributed peripherally in the thin layer of cytoplasm surrounding the highly vacuolate central region. The remainder are embedded in the denser cytoplasm between the embryo and the antipodals (fig. 3).

The endosperm growth is accompanied by an enlargement of the seed as a whole and a progressive absorption of the nucellus. These changes are il-

lustrated in figure 3 which shows a longitudinal section of a *H. jubatum* seed at 28 hours.

The cellular endosperm in a normal *H. jubatum* seed at 48 hours is illustrated in figure 5. At this stage the tissue consists of a single layer of cells, except in

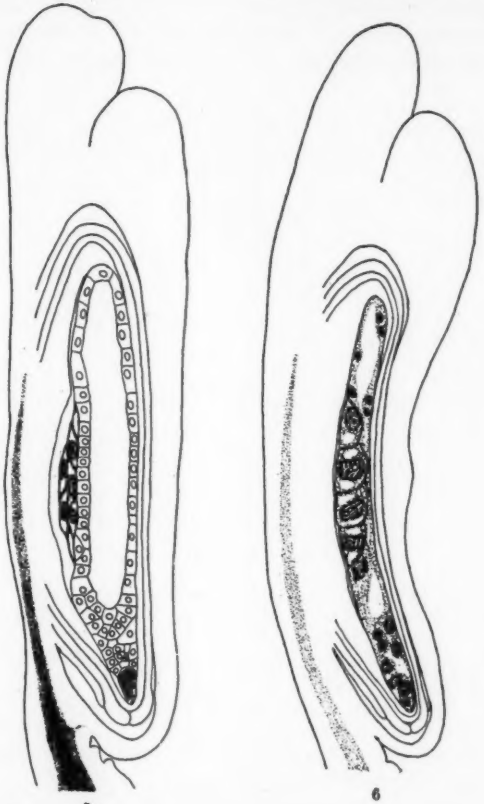

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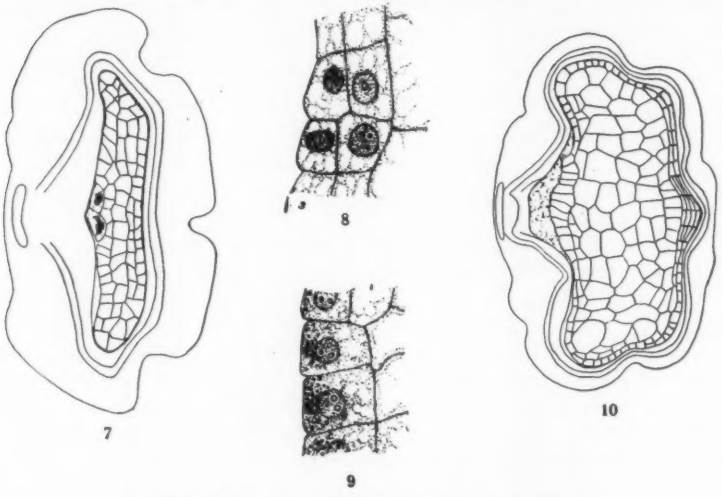
FIG. 5.—*H. jubatum*. Longitudinal section of a young caryopsis at 48 hours. The cellular endosperm surrounds a large central lumen. The apical cells of the nucellus are being digested leaving a cavity between it and the antipodals. ×33.

FIG. 6.—*H. jubatum*×*S. cereale*. Longitudinal section of a young caryopsis at 48 hours. Nuclei of varying sizes are present in the multinucleate endosperm. The antipodal cells are large and there is no evidence of digestion of the nucellus. ×33.

the pocket region adjacent to the embryo which is multilayered, surrounding a large, elongated, somewhat flattened, central cavity. Further growth occurs by cell division. The lumen of the endosperm becomes entirely filled with large, highly vacuolate cells by three days (fig. 7). Initial stages of food storage are

present at 82 hours and a large amount of starch is to be found in the central region of the endosperm at the four-day interval. By this time the peripheral cells have become differentiated as the aleurone layer. The aleurone cells are smaller than those of the main mass of the endosperm (fig. 8) and are highly vacuolate. The endosperm cells immediately opposite the vascular tissue of the ovary are exceptional. They are densely cytoplasmic and have the appearance of absorbing cells (fig. 9.) Active cell division continues and the endosperm in-



FIGS. 7 to 10.—*H. jubatum*. Stages in the development of the endosperm.

FIG. 7.—Transverse section through the mid-region of developing caryopsis at three days. ×33.

FIG. 8.—Highly vacuolate aleurone cells of a four-day endosperm. ×199.

FIG. 9.—Epidermal cells of a four-day endosperm from a region opposite the raphe. These cells are densely cytoplasmic and have the appearance of absorbing cells. ×199.

FIG. 10.—Median transverse section of a caryopsis at five days. The epidermal cells of the endosperm opposite the raphe are elongate. A viscous substance is present in the space between these cells and the remains of the nucellus. ×33.

creases in size (fig. 10). The highly vacuolate cells of the aleurone layer have become densely cytoplasmic and are covered with a thick cuticle at seven days (fig. 13). The outer layer of endosperm cells opposite the vascular tissue increases in size, becomes highly vacuolate and by seven days is becoming packed with storage materials. It does not become cutinized.

ENDOSPERM DEVELOPMENT IN THE HYBRID SEED

Behavior of the hybrid endosperm stands in marked contrast to that just outlined for the normal *H. jubatum* seed. The differences may be summarized as follows: (1) division of the primary endosperm nucleus is somewhat delayed; (2) mitotic division becomes disorderly, giving rise to irregular numbers

of nuclei which vary widely in size and form; and (3) the endosperm fails to become cellular even though it remains alive well beyond the time at which its counterpart in the *H. jubatum* seed attains this condition.

The delay in division of the hybrid primary endosperm nucleus is evident from the following facts. At four hours, two out of eight *H. jubatum* seeds examined already had binucleate endosperms, whereas the endosperm in all five hybrid seeds observed were still uninucleate. At six hours, three selfs had advanced to the four-nucleate condition, six were binucleate. Four out of six hybrid endosperms observed at this stage were uninucleate, the other two being binucleate. Nine in 13 *H. jubatum* endosperms were four-nucleate, the others being binucleate at eight hours. At this time, five hybrid endosperms in a total of 11 examined still possessed a single nucleus.

TABLE 1

Number of endosperm nuclei in H. jubatum and H. jubatum×S. cereale seeds.

TIME IN HOURS	*H. jubatum* SELFED			*H. jubatum×S. cereale*	
	NO. OF SEEDS	NO. ENDOSPERM NUCLEI		NO. OF SEEDS	MEAN NO. ENDOSPERM NUCLEI
		MODE	MEAN		
4	8	1	1.2	5	1.0
6	9	2	2.7	6	1.3
8	13	4	3.4	11	2.0
10	12	4	5.7	13	2.9
14	—			5	2.2
16	—			6	2.8
18	—			12	2.8
21	—			10	6.5
24	21	32	32.0	9	6.2
28	24	64	57.3	9	7.6
32	22	128	157.1	16	9.5
38	—			8	17.5
42	—			4	31.0
48	10	cellular		6	13.0
52	—			4	67.5
56	—			9	28.8
62	—			6	43.1

The further increase in number of nuclei in the hybrid endosperms is shown in the right hand column in table 1. Since the modal number of nuclei becomes progressively less well defined as age of the seeds advances the modal values have little significance and are not given. The means, however, show that in number of nuclei present after a given interval of time the hybrid endosperms fall behind those of *H. jubatum*, selfed.

Consideration of number of nuclei alone, however, gives a very incomplete picture of the character of the hybrid endosperms. Due to gross disturbances in mitotic behavior the nuclei also become highly variable in size and shape.

In table 2 is shown the proportion of hybrid seeds at successive 12-hour intervals up to 62 hours whose endosperms contain nuclei obviously abnormal in size. It will be noted that the frequency increases from 17 percent during the initial 12-hour period following pollination to 100 percent during the last interval (14 hours) at which observations were taken, ending at 62 hours. That

TABLE 2

Proportion of hybrid seeds having endosperms containing nuclei of abnormal size.

TIME INTERVAL IN HOURS	NUMBER OF SEEDS		PERCENTAGE ABNORMAL
	TOTAL	ENDOSPERM NUCLEI ABNORMAL	
0–12	35	6	17
13–24	42	19	45
25–36	25	16	64
37–48	18	11	61
49–62	19	19	100

is to say, all the hybrid endosperms, sooner or later, become abnormal in this respect.

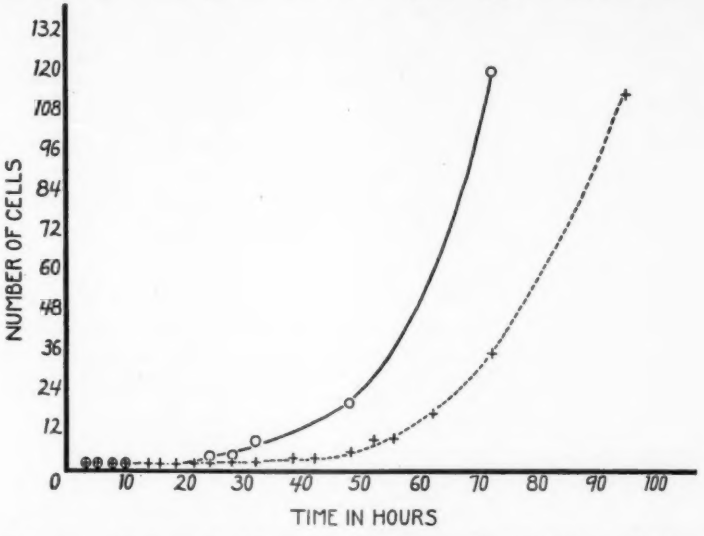
The diversity in number, shape and size of nuclei arising in the hybrid endosperms is very great. Figure 4, based on a 28-hour seed, and figure 6, showing a 48-hour seed, illustrate how wide the departure may be from the normal condition at these times (*cf.* figs. 3 and 5). All the endosperm nuclei shown in figure 4 are excessively large and instead of being distributed peripherally throughout the whole endosperm they all lie between the embryo and the antipodals. A more uniform distribution is seen in figure 6, but here the nuclei in the vicinity of the embryo are excessively large in contrast with those in the distal portion of the tissue. One nucleus in the former group is dumbbell-shaped. Variously malformed nuclei are frequent.

Abnormal mitotic behavior sometimes occurs in division of the primary endosperm nucleus. An anaphase of such a first division is shown in figure 14. It will be noted that due to several chromosomes bridging the spindle the separation of the two daughter groups of chromosomes is not complete. Such chromosome bridges may lead to dumbbell-shaped interphase nuclei like that shown in figure 15. These compound nuclei may then enter another division cycle (fig. 16) during which a greatly increased number of chromosomes appears. Giant nuclei (fig. 17) thus arise. Figure 18 shows a metaphase plate from a 52-hour endosperm on which 105 chromosomes are distributed. This number is three times the value (35) expected in the endosperm nucleus of a hybrid between *H. jubatum* (n=14) and *S. cereale* (n=7). The increase in chromosome number in the abnormal hybrid nuclei, however, does not always proceed in multiple series. Aneuploid numbers also arise frequently as a result of various kinds of mitotic irregularities. These nuclear disturbances are

cumulative and, as stated above, eventually appear in the endosperms of all the hybrid seeds.

EMBRYO GROWTH

Development of the young *H. jubatum*×*S. cereale* embryo is comparatively regular in sharp contrast to the frequently radical derangement of the accompanying endosperm. Rate of embryo growth up to 96 hours, in comparison with that of the normal *H. jubatum*, may be seen from the data given in table 3 and plotted in text figure 1. Most of the hybrid zygotes divide between 18



TEXT FIG. 1.—Early growth of the embryo following the matings *H. jubatum*, selfed (continuous line) and *H. jubatum*×*S. cereale* (broken line).

and 24 hours. This is apparently a few hours later than in *H. jubatum* for which, however, the data are less complete over this period. At 24 hours the average number of cells in the embryo of the *H. jubatum* seed is four as against 1.8 for the hybrid. From this time forward to 96 hours the hybrid embryos attain a given size about one day later than in the control.

When compared with the development of the normal embryo (fig. 11) differentiation of the hybrid embryo appears to be almost normal, as illustrated in figure 12. There is a tendency from four days onward for the hybrid embryos to become somewhat more elongated than the controls probably as a result of less compression in this direction due to the absence of a cellular endosperm.

It is interesting to note also that up to 62 hours, beyond which time the necessary data were not taken, there seems to be little relationship between size of the embryo and the nuclear condition of the endosperm so long as the latter

tissue is still alive. For example, at 56 hours, four of the nine seeds recorded in table 3 for that time had 8-celled embryos. The endosperms associated with these embryos showed the following nuclear conditions, respectively: 32 large; 4 large, 8 small; 42, large and small; 48, large and small; 2 very large. The endosperms in three seeds with 10-celled embryos possessed nuclei as

TABLE 3

Number of cells in H. jubatum and H. jubatum×S. cereale hybrid embryos.

TIME IN HOURS	*H. jubatum*, SELFED		*H. jubatum×S. cereale*	
	NO. OF SEEDS	MEAN NO. CELLS IN EMBRYO	NO. OF SEEDS	MEAN NO. CELLS IN EMBRYO
4	8	1	5	1
6	9	1	6	1
8	13	1	11	1
10	12	1	13	1
14	—	—	5	1
16	—	—	6	1
18	—	—	12	1
21	—	—	10	1.7
24	21	4	9	1.8
28	24	4.4	9	2.7
32	22	8.5	16	2.3
38	—	—	8	3.5
42	—	—	4	3.5
48	10	20	6	5.5
52	—	—	4	9
56	—	—	9	9.1
62	—	—	6	17
72	12	120	11	35
96	—	—	5	113

follows: 8 large, 16 small; 4 large, 8 small; 8 large. One seed with a 12-celled embryo possessed about 80 large and small endosperm nuclei. Possibly beyond this period the number and kind of nuclei present in the endosperm may become critical for the growth of the embryo but in the earliest stages of seed development, at least, this is not the case.

BEHAVIOR OF THE ANTIPODALS

The antipodals are a prominent constituent of the mature embryo sac of *H. jubatum*, as in other species of Gramineae. The number of antipodals in *H. jubatum* at fertilization is usually 15, and they occupy about one-quarter of the space in the embryo sac. The tissue is situated initially at the chalazal end of the sac (fig. 1), but it soon becomes lateral as a result of growth of the endosperm beyond it (fig. 2). In the latter position the antipodals lie on the funicular side of the ovule opposite the vascular bundle. They are, therefore, in the path which nutrient materials would be expected to follow in entering the endosperm.

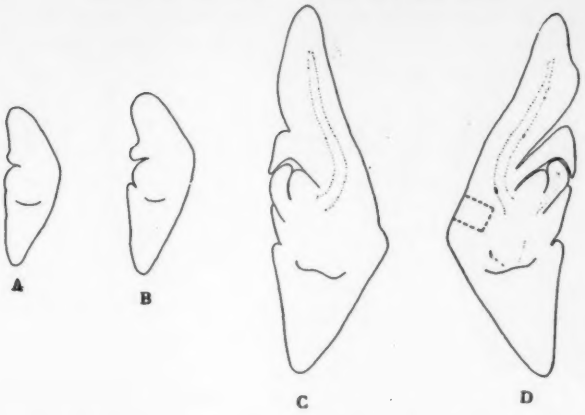


FIG. 11.—*H. jubatum*. Stages in the development of the embryo at *A*, five; *B*, six; *C*, nine and *D*, 11 days respectively. ×33.

Parallel with normal fertilization in *H. jubatum* the antipodals enlarge about six-fold. A proportionate increase in volume of the embryo sac also occurs at this time. The antipodal nuclei, likewise, increase greatly in size. The enlarged antipodal cells become increasingly vacuolate (figs. 1, 2 and 3) and reach their maximum expansion at about 28 hours. No increase in cell number occurs.

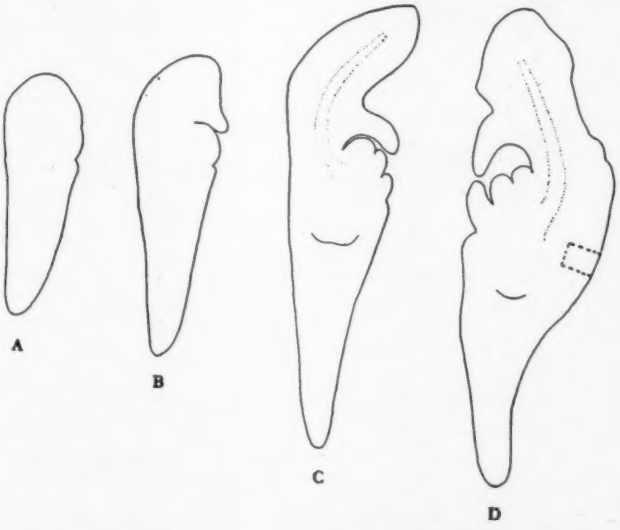


FIG. 12.—*H. jubatum*×*S. cereale*. Stage in the development of the embryo at *A*, five; *B*, six; *C*, nine and *D*, 11 days respectively. ×33.

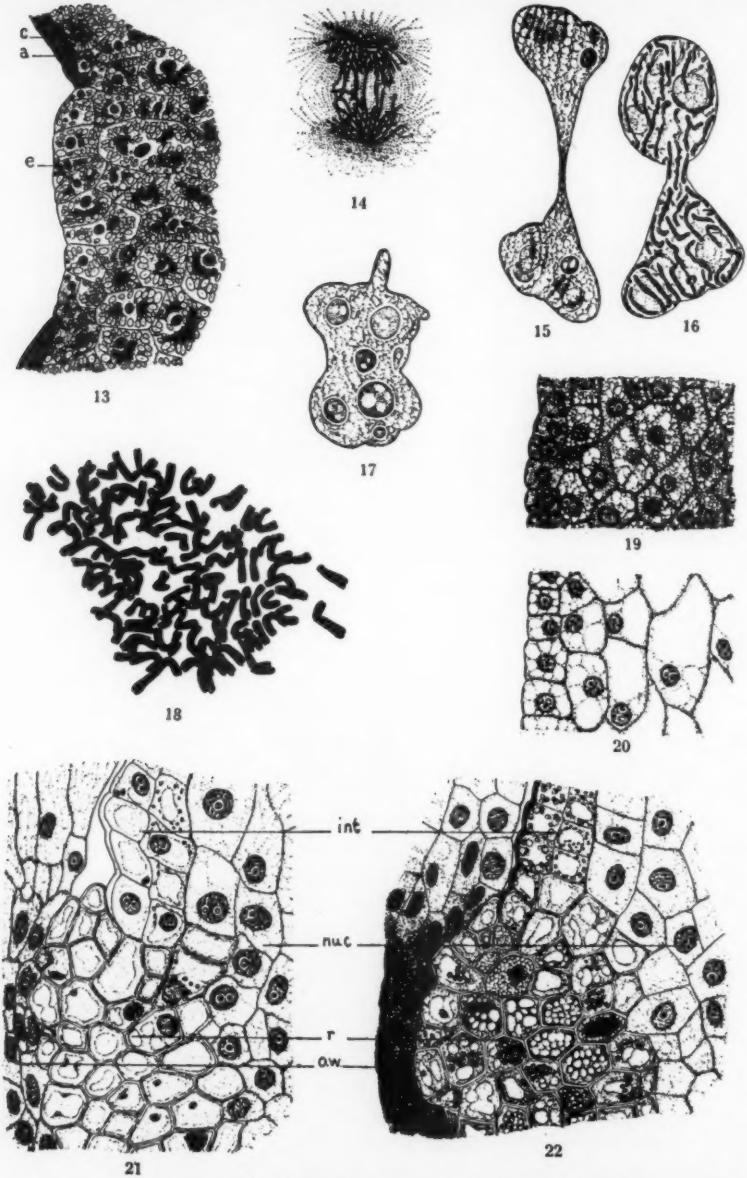


FIG. 13.—*H. jubatum*. Median transverse section of a portion of the endosperm opposite the raphe at 7 days. The epidermal cells in the mid-region (e) are thin walled and contain storage

From 28 hours onward the antipodals shrink steadily until at 48 hours the tissue is reduced to a flattened group of cells pressed between the nucellus and the now cellular endosperm (fig. 5). Disintegration continues until, at 72 hours, only fragments remain.

Following fertilization in the *H. jubatum* ovule by *S. cereale* sperm, on the other hand, the course of development of the antipodals is very different. The rapid, initial enlargement of the cells and nuclei observed in *H. jubatum* selfed at fertilization does not occur. In contrast the cytoplasm remains dense, or only small vacuoles appear in a few cells. A correspondingly limited increase in embryo sac size takes place. From this time onward the antipodals enlarge slowly, although at 28 hours they are still small and dense relative to their counterparts in the normal *H. jubatum* seed (fig. 4). At this time some cells undergo mitotic division, and this activity continues to about 72 hours. Regression of the tissue begins some hours later, and by 96 hours breakdown is clearly apparent. Further data on the antipodals are presented in a following paper in which the relationship of this tissue to endosperm behavior is considered in detail.

THE IMMEDIATE CAUSE OF SEED COLLAPSE

The hybrid seed collapses promptly following breakdown of the last traces of endosperm. The immediate cause of failure appears to be starvation associated with hypofunction, and, eventually, disintegration of the endosperm. It was pointed out above that the hybrid embryo, although retarded in growth,

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materials. The small aleurone cells (a) on either side are densely cytoplasmic and a dense cuticle (c) covers the outer surface. ×69.

FIGS. 14–18.—*H. jubatum*×*S. cereale*. Stages of mitosis in the endosperm and the formation of giant nuclei.

FIG. 14.—Anaphase of the division of the primary endosperm nucleus with chromosome bridges at 8 hrs. ×324.

FIG. 15.—Dumb-bell shaped interphase nucleus at 8 hrs. ×324.

FIG. 16.—Prophase stage of division of a dumb-bell shaped nucleus at 16 hrs. ×324.

FIG. 17.—Large nucleus in a 32-hour endosperm. ×324.

FIG. 18.—Polar view of a metaphase with 105 chromosomes. ×648.

FIG. 19.—*H. jubatum*. Detailed sketch of that portion of embryo outlined in figure 11 d. The cells are densely cytoplasmic. Granular materials are stored in the uniformly dense cytoplasm of the epidermal cells. ×174.

FIG. 20.—*H. jubatum*×*S. cereale*. Detailed sketch of that portion of the embryo outlined in figure 12 d. The cells are large and highly vacuolate. There is no evidence of storage materials in the epidermal layer. ×173.

FIG. 21.—*H. jubatum*. Portion of the raphe and adjacent tissues at 7 days; *r*—cells of the raphe with thick walls and gelatinous contents; o.w.—ovary wall with vacuolate cells and normal nuclei; int.—integument with transparent cuticle; nuc.—nucellus with normal resting nuclei. ×324.

FIG. 22.—*H. jubatum*×*S. cereale*. Portion of raphe and adjacent tissues at 7 days; r—the cells of the raphe have thick walls and are filled with a dense gelatinous material in which is embedded granular substances; o.w.—ovary wall—the nuclei of the cells opposite the raphe are disintegrating and the cytoplasm is very dense; int.—integument with dense cuticle. The cells are packed with granular materials; nuc.—nucellus with normal resting nuclei. ×324.

differentiates in the usual way. Formative processes in this tissue, therefore, are not visibly impaired by the hybridity. There is, however, a conspicuous difference in the nutrition of *H. jubatum* and *H. jubatum*×*S. cereale* embryos. This difference is illustrated in figures 19 and 20 which are based upon 11-day old seeds of the two respective classes.

The stage of development attained by the 11-day *H. jubatum* embryo represented in figure 11 D and the *H. jubatum*×*S. cereale* embryo drawn in figure 12 D is nearly the same, the hybrid being only slightly less advanced. The cellular condition prevailing in these embryos is shown in figures 19 and 20, which represent at an increased magnification a portion of the scutellum indicated by broken lines in figures 11 D and 12 D, respectively. The cyto-

TABLE 4

Frequency of collapsing hybrid seeds at stated intervals following fertilization.

TIME IN DAYS	NUMBER OF SEEDS		PERCENTAGE COLLAPSING
	TOTAL	COLLAPSING	
3	16	0	0
4	20	2	10
5	23	1	4
6	23	0	0
7	35	5	14
8	8	2	25
9	7	2	29
10	7	4	57
11	12	9	75
12	—	—	—
13	10	10	100

plasm in the scutellar cells of the control embryo is dense and finely vacuolate. The outer layer of cells is especially rich in cytoplasm and contains considerable food material in granular form. In contrast, the cells in the corresponding region of the hybrid embryo are larger and much more highly vacuolate (fig. 20). The total mass of cytoplasm in these cells appears to be low relative to that in the *H. jubatum* cells. The cells of the outer layer, likewise, are highly vacuolate and are entirely lacking in granular reserve material. This starved appearance characterizes the entire hybrid embryo.

Undernourishment of the hybrid embryo is doubtless a result of malfunctioning of the associated endosperm. Development of the latter tissue is radically disturbed by mitotic irregularities, as described earlier. In spite of its abnormal condition, the hybrid endosperm may continue to support embryo growth for several days. The plane of nutrition declines, however, until the low level represented in figure 20 is reached. This is the prelude to collapse of the seed.

Five hybrid seeds were observed, varying in age from four to 11 days, in which cell division in the embryo had ceased although the embryo was still

intact. The endosperm had disappeared in two of these seeds and comprised a very small amount of tissue in the remaining three. Endosperm activity in the later cases had apparently dropped below the level at which growth of the embryo can proceed. The endosperms had entirely broken down in 31 other seeds, four to 13 days of age, and the embryos were disintegrating. These seeds and the five mentioned above were collapsing.

THE OVARY AND CERTAIN ACCESSORY TISSUES OF THE SEED

The *H. jubatum* ovary contains a single vascular bundle which enters at the base and extends about half-way to the apex in the mid-region of the broad, somewhat flattened ventral wall as shown in figure 1. The ovule is attached to the inner surface of the ovary wall, its base being directly opposite the apex of the vascular bundle. The basal cells of the ovule which constitute the raphe serve to conduct nutrient materials into the developing seed. This conducting tissue is not conspicuously differentiated as such but is continuous with the nucellus on its inner side and with the ovary wall outwardly.

The antipodal cells in the initial stages of seed development are immediately adjacent to that portion of the nucellus directly opposite the raphe and very probably play an important role in transmitting food materials to the endosperm during the period in which the latter tissue is in the free nuclear condition. These cells break down shortly after the endosperm becomes cellular and from that time on the principal absorbing surface of the endosperm appears to be the portion adjacent to the above mentioned conducting tissue. The outer layer of endosperm cells in older seeds comprises the aleurone which is well differentiated at five days. The aleurone layer in typical form does not extend across the absorbing region but is replaced here by elongated cells (fig. 10) whose main axis is directed toward the interior of the endosperm, doubtless facilitating the passage of nutrients into this tissue.

The nucellus is progressively absorbed as the endosperm develops so that at five days a very small portion only remains directly opposite the vascular bundle (figs. 1, 3, 7 and 10). This part of the nucellus, which functions as conducting tissue, is digested on the side toward the endosperm but is maintained at a functional level for about five days by cell division in the basal region. Lying between it and the endosperm in five-day seeds is a narrow cell-free gap filled with a viscous substance probably of a nutritive character (fig. 10).

The nuclei of the cells of the raphe in *H. jubatum* seeds show early signs of disintegration about five days after pollination and at seven days such nuclei as remain are uniformly dense and there is little evidence of a chromatin-linin network. The cells at this stage have thick walls and the cytoplasm has a gelatinous appearance with a dense peripheral layer and a less dense mid-region (fig. 21). As the seed approaches maturity these cells become packed with storage materials of a mucilaginous character.

The outer integument becomes flattened between the inner integument and the ovary wall, due to the rapid expansion of the endosperm, collapses and disintegrates within the first four or five days after pollination. The cells of

the inner integument enlarge somewhat, the cytoplasm becomes transparent and the nuclei have a more or less granular appearance (fig. 21). The exposed surface of the outer layer of cells becomes coated with a thin transparent cuticle. The cells of the inner integument are much flattened between the single layer of nucellar cells and the ovary wall by the expansion of the endosperm as the seed approaches maturity.

Histologically, the course of early development of the *H. jubatum*×*S. cereale* hybrid seeds, aside from the antipodals and the endosperm, is not conspicuously different from that just outlined for *H. jubatum*. The ovary increases in size following fertilization, although less rapidly than in the control, and probably lags behind the latter in size at all ages. The nucellus is gradually absorbed, and only in the region opposite the vascular bundle is a difference in its behavior to be noted. As digestion of the tissue proceeds in association with the enlarging endosperm the nucellus is not renewed by cell division in the basal portion of the absorbing region. Throughout the life of the hybrid seed, therefore, the role of the nucellus is entirely passive. There is no accumulation of nutritive material between the endosperm and the nucellus as occurs in *H. jubatum* selfed.

The outer integument of hybrid seeds becomes crushed during the early course of seed development. The nuclei of the cells of the inner integument begin to disintegrate and the cytoplasm takes on a uniform gelatinous appearance about three days after pollination. A dense cuticle is formed on the exposed surface of the integument and the cells become packed with storage materials. The materials in the outer layer of cells at seven days have the appearance of oil droplets as contrasted with the fine granules present in the inner layer (fig. 22).

The nuclei of the cells of the raphe likewise show evidence of breakdown at three days and have completely disintegrated at five days, at which time the cytoplasm has a gelatinous appearance, and there is some accumulation of storage materials. The cell walls become thickened and this accumulation continues so that by seven days the cells are packed with storage materials of various kinds (fig. 22). This series of changes is similar to that which occurs in *H. jubatum* seeds but it takes place earlier. Apparently the alteration of this tissue does not interfere with the movement of nutrients into the endosperm since considerable storage takes place in the normal seed after it is completed. Likewise its premature onset in hybrid seeds does not appear to be directly related to collapse. The early and sudden breakdown of *H. sativum*×*S. cereale* seeds may occur in advance of any visible change in the raphe, whereas such transformation is normal in somewhat older common barley (*H. vulgare*) seeds just as in *H. jubatum*.

The histological differences just related as characterizing certain of the accessory tissues of the seed and the ovary following the *H. jubatum*×*S. cereale* mating probably have little relation to collapse of the seed. They are important, however, in illustrating what appears to be a significant feature of failure in this particular case, namely, the relatively weak development of the

entire caryopsis including the hybrid seed within. No part of the structure, leaving aside the antipodals which become hyperplastic, attains the degree of development following the cross that is found in the corresponding tissue of the normal *H. jubatum* seed.

DISCUSSION

The ultimate failure of the *H. jubatum*×*S. cereale* seed may be attributed to starvation. The entire structure appears to be undernourished. The impaired nutritive condition is conspicuously shown in the embryo. This fact is significant because the embryo is normally formed and potentially functional. That is to say, differentiation occurs in the usual manner during the life of the seed, even though the rate of growth is reduced. Furthermore, the zygote is capable of development beyond the most advanced stage reached *in situ*. The latter fact is attested by the successful rearing of a hybrid plant from an embryo excised from a seed in advance of collapse and cultivated on a synthetic medium (BRINK, COOPER and AUSHERMAN, in press).

It is a noteworthy fact that the embryos resulting from hybrid fertilization, in general, undergo normal differentiation even though other tissues in the seed may depart widely from the normal course of development. The hybrid embryo may be relatively undersized for its age, but it remains alive as long as the seed continues growth, and differentiates in a regular manner. BOYES and THOMPSON (1937) have emphasized the contrast in this respect between embryo and endosperm following certain interspecific matings in Triticum. The observations of workers on other species are in agreement. One may conclude, therefore, that the conditions necessary for development of the embryo into a normal form are not wanting in these hybrid seeds. Growth, as distinguished from morphallaxis, however, appears to be more or less reduced at all stages and ceases as the seed approaches collapse. It would appear that the hybrid embryo can utilize the nutrients it receives; but the supply is limited. This leads, in the extreme case, to starvation. On these grounds the search for an explanation of seed collapse in the *H. jubatum*×*S. cereale* mating was directed to the nutritive mechanism.

The tissue which occupies the central position in nourishment of the seed is the endosperm. The extensive morphological literature bearing on this question has been critically surveyed by SCHNARF (1929), and will not be reviewed here. The present investigators have shown more recently how the findings on seed failure following inbreeding in *Medicago sativa* (BRINK and COOPER 1940) and interspecific hybridization in Nicotiana (COOPER AND BRINK 1940) provide additional support for this view. These latter studies have yielded evidence indicating that not only does the endosperm absorb food materials and transmit them to the embryo but also that certain early post-fertilization structural changes of primary significance for the distribution of nutrients within the seed are under its control.

The endosperm of the Gramineae, unlike that of most other families of flowering plants, persists to maturity of the seed in which it forms a massive

storage tissue. The writers are unaware of any evidence, however, indicating that persistence of the endosperm, in itself, is a factor of any significance for the type of seed failure with which we are here concerned. Several students of interspecific hybridization in the cereals have observed that the germinative power of the seed is correlated with plumpness of the mature endosperm (*cf.* KIHARA and NISHYAMA 1932; WAKAKUWA 1934). THOMPSON and CAMERON (1928) point out also that differential germination incident upon degree of shrivelling of the seed may play an important part in the non-appearance of certain expected genotypes in segregating families derived from wide cereal crosses. Obviously, therefore, the condition of the endosperm in the ripe seed is a significant aspect of the general problem of hybrid incompatibility in this group of plants. The important point in the present connection, however, is that, as KIHARA and NISHIYAMA (1932), WAKAKUWA (1934) and BOYES and THOMPSON (1937) have shown, the abnormal condition of the mature hybrid endosperm in the Gramineae has its origin in developmental disturbances which may appear soon after fertilization. Thus, the impairment in seed development following wide crosses in the cereals, which have a persistent endosperm, traces back to the same stages at which disfunction of the endosperm may initiate seed failure in the much more numerous species in which the tissue is of short duration. From this point of view, the fact that the cereal endosperm eventually becomes an important storage organ is incidental. The tissue also has a basic function in the morphogenesis and early growth of the seed; and, in the absence of evidence to the contrary, its significance in the Gramineae in these respects may be assumed to be neither greater nor less than that in other Angiosperms.

A remarkable feature of the endosperm in all *H. jubatum*×*S. cereale* seeds observed is the abnormal mitotic behavior of the nuclei. Chromosome distribution becomes highly irregular, leading to widely varying numbers of nuclei of diverse sizes and shapes. Division of the primary endosperm nucleus, sometimes occurring within two hours after fertilization, may be affected, or the first evidence of disturbance may appear at varying times thereafter up to 60 hours. Furthermore, the tissue never becomes cellular, a change which takes place in normal *H. jubatum* seeds at about 48 hours. This extraordinary nuclear behavior in the endosperm was reported by KIHARA and NISHIYAMA (1932) in the cross *Avena strigosa*×*A. fatua* and has also been described by BOYES and THOMPSON (1937) in *Triticum durum*×*T. vulgare*, *T. dicoccum* var. Khapli× *T. vulgare*, *T. spelta*×*T. monococcum* and reciprocal. We find that it also characterizes the endosperm of the short-lived *Hordeum vulgare*×*Secale cereale* seed. These data suggest that the phenomenon may be of wide occurrence following distant crosses in the Gramineae. It has not been observed following interspecific hybridization in the other families which have been used in studies of this kind, namely, the Onagraceae (RENNER 1915), Labiatae (MÜNTZING 1930), Solanaceae (SATINA and BLAKESLEE 1935; COOPER and BRINK 1940) and Leguminoseae (LEDINGHAM 1940).

If it is true that the endosperm plays the primary role in early development

of the young seed, as suggested above, it is not difficult to understand why the *H. jubatum*×*S. cereale* hybrid fails. The highly unbalanced genic conditions in the endosperm nuclei resulting from irregular chromosome distribution must greatly impair the functional capacity of the tissue. An early result is a reduced supply of food materials to the seed and progressive starvation of the otherwise normal embryo. Observations on seeds approaching collapse show that even a small amount of endosperm tissue suffices to keep the seed alive. But when the last traces of the tissue disintegrate the seed promptly breaks down. These facts indicate that the normal endosperm of *H. jabutum* provides a considerable margin of safety for seed formation. That is to say, a hybrid embryo may attain a given degree of early development in association with a considerably weaker endosperm than accompanies a normal embryo at the same stage.

The abnormal mitotic behavior of the endosperm nuclei, although not known to occur following other species crosses, is such a frequent and positive feature of seed failure in the Gramineae as to constitute a major aspect of the problem in this family. In view of the non-appearance of the phenomenon in other matings which have been studied one might suspect that the observed nuclear irregularity has its basis in some characteristic of the Gramineae not common to flowering plants in general. Such indeed appears to be the case. The evidence obtained on *H. jubatum*×*S. cereale* seeds points to the antipodals as the source of the unusual endosperm behavior. The antipodals form a very prominent tissue in the mature embryo sac of the Gramineae. They enlarge greatly at fertilization and undergo a series of morphological changes during the period of free-nucleate endosperm development suggesting intense secretory activity. This behavior, while not unique among the angiosperms, is uncommon, the antipodals usually regressing quickly following syngamy (SCHNARF 1929). As described briefly above and considered more fully in a following paper, the *H. jubatum* antipodals react very differently when *S. cereale* sperm are substituted for *H. jubatum* sperm in fertilization; and the difference is readily measurable before the primary endosperm nucleus divides. Advent of rye sperm to the *H. jubatum* embryo sac fails to arouse the antipodals to normal activity, and they tend to remain small and densely staining. Division of the primary endosperm nucleus, on the other hand, is only slightly delayed after hybrid fertilization, and further development of this tissue proceeds in association with the comparatively quiescent antipodals. It is evident from the location of the antipodals in the nutrient stream between the vascular bundle and the endosperm that the latter may become undersupplied with certain substances essential to nuclear growth and division, and that mitotic irregularities might thus arise.

In the light of these findings a re-examination of the antipodals in other cereal crosses in which abnormal nuclear behavior characterizes the endosperm would be helpful. KIHARA and NISHIYAMA (1932) refer briefly to the tissue in their study of seed development following reciprocal crosses between *Avena fatua* and *A. strigosa* and do not note any unusual behavior. It seems not unlikely, however, that differences present in the other hybrid cereals studied

have been overlooked. The true situation in *H. jubatum*×*S. cereale* seeds became apparent when the volume changes in the antipodals and embryo sac were measured as development proceeded. These data have not been reported for the other cereal hybrids which have been studied.

We have not found evidence of overgrowth in the maternal tissues of the aborting *H. jubatum*×*S. cereale* seed. This, like the abnormal behavior of the endosperm nuclei just discussed, is a departure from the course of development leading to failure in Nicotiana, and probably various other, hybrid seeds (*cf.* COOPER and BRINK 1940). It raises again the question which THOMPSON (1940) has recently posed, whether impaired functioning of the endosperm is directly responsible for collapse of the hybrid seed rather than indirectly, as we have inferred in Medicago (BRINK and COOPER 1940) and in various Solanaceous species crosses (COOPER and BRINK 1940; BRINK and COOPER 1941). The evidence we obtained pointed to a secondary factor being involved in seed failure, namely, overgrowth of maternal tissue adjacent to the endosperm. The hybrid endosperm was found to be inherently weak so that the maternal tissues outgrew and starved it. The term "somatoplastic sterility" was applied to the phenomenon as descriptive of the endosperm-maternal tissue unbalance leading to seed failure.

There is no evidence of such competition in the *H. jubatum*×*S. cereale* caryopsis. The nuclear disorganization present is alone sufficient, however, to cause breakdown of the endosperm and death of the seed. Although fundamentally like the cases described in Nicotiana in that the main factor in failure is disfunction of the endosperm, this example does not conform to the pattern there found so far as overgrowth of the maternal tissue is concerned. What is the basis of the difference? Furthermore, in view of this exception, is there justification for considering that a developmental unbalance, arising primarily from genetic diversity, between endosperm and associated maternal tissue is a general relation in seed failure following distant crosses? Additional data are needed before either of these questions can be answered with confidence. Meanwhile certain considerations which appear important for a further analysis of the problem may be set down.

Basically, collapse of the *H. jubatum*×*S. cereale* seed appears to stem from failure of the hybrid endosperm to arouse the *H. jubatum* antipodals to full functional activity. This fact raises doubt concerning the extent to which the findings on seed failure in the Gramineae may be applicable to other flowering plants in view of the infrequency of species in which the antipodals persist after fertilization. The extent to which the antipodals may occasion exceptional endosperm behavior in the cereals in general cannot be stated without further study but the possibilities should not be underestimated. Intercalation in a diploid maternal sporophyte-triploid endosperm system of an active haploid antipodal (gametophytic) tissue may well give rise to a set of physiological relations differing markedly from that ordinarily obtaining within the seed.

Whether competition between maternal tissues and endosperm ever occurs following species hybridization in the Gramineae, giving rise to the condition we have described as somatoplastic sterility in Medicago and Nicotiana must

remain an open question for the present. Possibly its absence in the *H. jubatum* ×*S. cereale* seed is due to the relatively week activity of the endosperm from the start. If the hybrid endosperm affects the antipodals only feebly its influence beyond these cells in stimulating development of the maternal tissues of the seed may be even less.

WAKAKUWA's (1934) data on seed size in Triticum are of interest in this connection. He measured length and width (maximum values) of the seeds at intervals from 15 to 96 hours after pollination following a series of interspecific hybrid matings and compared the results with those from the respective normally fertilized maternal parents at corresponding ages. We have computed the product of the two values which WAKAKUWA gives, as providing a simple although rough basis for comparison. The value for the normal matings is reduced to 100 in each case. The size of seed of each hybrid is then expressed as a percentage of the value for the respective maternal parent. The results thus obtained at 15 hours, which is shortly after fertilization, are as follows:

T. spelta	100
T. spelta×*T. polonicum*	127
T. spelta×*T. aegilopoides*	115
T. polonicum	100
T. polonicum×*T. spelta*	125
T. polonicum×*T. aegilopoides*	110
T. aegilopoides	100
T. aegilopoides×*T. spelta*	139
T. aegilopoides×*T. polonicum*	108

It will be noted that in all cases the hybrids exceed the control seeds in size. WAKAKUWA obtained similar results at 24 hours. Only at later stages did certain of the hybrids fall behind their controls. WAKAKUWA's observations on the rate of endosperm development are somewhat inconsistent. It appears, however, from the data given on time of cell wall formation that this tissue lags behind the control in all hybrids except those in which *T. spelta* served as the pistillate parent. *T. spelta*×*T. polonicum* and *T. spelta*×*T. aegilopoides* are reported as beginning to form cell walls in the endosperm at 48 hours, as in normal *T. spelta*. The hybrid embryos throughout, however, were found to be smaller than their respective controls at 120 hours.

WAKAKUWA's data suggest that, with the two possible exceptions noted, endosperm and embryo in the Triticum hybrids are more or less retarded in early development whereas the maternal tissues of the seed increase in size more rapidly than in the controls. Whether this apparent overgrowth of the maternal tissue reacts unfavorably on the endosperm and thus depresses seed development is worthy of further study.

SUMMARY

Failure of the hybrid seed to develop to a germinable condition following the mating *Hordeum jubatum*♀×*Secale cereale*♂ is a result of starvation arising from irregular development and, eventually, complete breakdown of the

endosperm. Division of the hybrid primary endosperm nucleus may be somewhat delayed; mitotic division becomes disorderly, giving rise to abnormal numbers of endosperm nuclei varying widely in size and form; and the hybrid endosperm fails to become cellular as in normal *H. jubatum*.

The hybrid embryo is retarded in early development but differentiates normally. The embryo shows pronounced evidence of starvation in seeds about to collapse.

The antipodals, comprising about 15 cells lying opposite the vascular bundle, form a massive tissue in the mature *H. jubatum* embryo sac. The antipodals normally persist during the free-nucleate endosperm stage during which they undergo a striking series of changes in size and character.

The advent of *S. cereale* sperm in fertilization evokes a much less pronounced initial response in the antipodals than follows normal syngamy and the subsequent behavior of the antipodals is radically changed. The irregular mitotic behavior of the hybrid endosperm is interpreted as a secondary effect proceeding from the associated antipodals.

Attention is called to the possibility that seed failure following distant hybridization in the Gramineae generally may involve developmental changes not encountered in most other flowering plants because of the intercalation in the nutrient stream between maternal sporophyte and endosperm in this family of a highly developed antipodal tissue which functions during early post-fertilization stages.

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THE ANTIPODALS IN RELATION TO ABNORMAL ENDOSPERM BEHAVIOR IN HORDEUM JUBATUM×SECALE CEREALE HYBRID SEEDS[1]

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INTRODUCTION

GROSS mitotic irregularities in the endosperm are a characteristic feature of seed development following certain interspecific matings in the Gramineae. KIHARA and NISHIYAMA (1932) reported this behavior for the cross *Avena strigosa*×*A. fatua*, and it has been observed in other cereal hybrids since by several investigators. The seed resulting from mating *Hordeum jubatum* (squirrel-tail barley) with *Secale cereale* (rye), as described in the preceding paper, is a case in point. Abnormal chromosome distribution may occur at the first nuclear division following triple fusion; or the initial irregularity may appear at an early mitosis thereafter. A result of this behavior is that the endosperm nuclei come to vary widely in size and chromosome number; and the hybrid endosperm fails to become cellular as in normal *H. jubatum*. Eventually the ill-developed endosperm tissue ceases growth and the hybrid seed breaks down six to 13 days after fertilization.

The early and invariable occurrence of abnormal mitosis in the Hordeum ×Secale endosperms might lead one to think that the phenomenon is an immediate effect of the cross and the basic cause of the seed failure. This explanation rests on the assumption that the two diverse genomes, when combined, impair directly the capacity of the hybrid nucleus to divide in the usual manner. There are certain other clearly established facts, however, which are not reconcilable with such an interpretation. In the first place the embryo, which is likewise a hybrid structure, shows regular mitosis. Secondly, a *H. jubatum*×*S. cereale* plant has been reared to the adult stage from an embryo excised from the immature seed and cultivated on an artificial nutrient medium (BRINK, COOPER and AUSHERMAN, 1944). One would not expect the hybrid zygote to be capable of such extended development if the combined Hordeum and Secale genomes tend to upset the mitotic mechanism. A third fact casting doubt on this explanation is that mitotic irregularities eventually may appear also in the antipodals following fertilization of the squirrel-tail barley by rye. The antipodals are not of hybrid origin, of course, but carry a haploid complement of *H. jubatum* chromosomes only.

The above considerations make it probable that the mitotic abnormalities in the hybrid endosperm are of secondary origin. Since the nuclear disturbances

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are irreversible, however, development of the endosperm is permanently handicapped once the chromosome distribution is upset; and the impairment of this tissue leads ultimately to collapse of the seed, as described in the preceding article. On this view an explanation of seed failure following the cross is to be sought in the conditions underlying early growth of the hybrid endosperm. The primary endosperm nucleus may divide irregularly within an hour or so

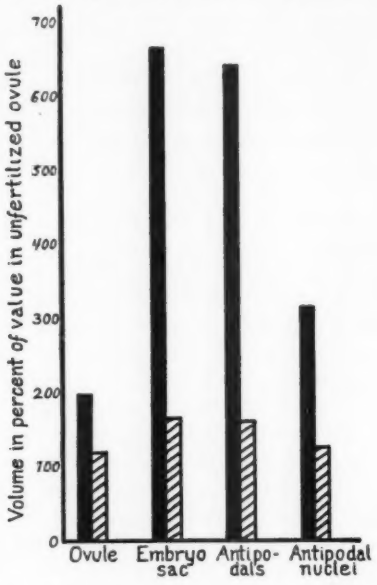

FIG. 1.—Relative volumes at fertilization of the ovule, embryo sac, antipodals and antipodal nuclei following the matings *Hordeum jubatum*, selfed (solid bar), and *H. jubatum*×*Secale cereale* (crosshatched bar). Volumes of the four respective tissues in the unfertilized ovule are taken as 100.

after fertilization. Accordingly, search for a possible cause of the aberrant endosperm behavior was directed to the embryo sac during the brief period between presentation of the two male gametes to the egg and polar nuclei, respectively, and the first endosperm division.

CHANGES IN THE OVULE AT FERTILIZATION

Since it was desired to determine whether significant effects on the ovule of the mating with rye could be observed before mitosis in the endosperm had begun, ovules were sought at two hours and four hours after pollination in which fertilization had occurred but in which division of the primary endosperm nucleus had not yet begun. Four such ovules from the *H. jubatum*×*S. cereale* mating and a like number from *H. jubatum* selfed were found in the

material at hand. These eight ovules had been fixed at four hours after pollination. Three unfertilized *H. jubatum* ovules taken at two hours after pollination were available for comparison.

The volumes of these 11 ovules and of their contained embryo sacs, antipodals and antipodal nuclei were determined. These values were computed

TABLE 1

Volume of the Hordeum jubatum ovule and of certain contained structures at (1) the mature embryo sac stage, (2) after self-fertilization and (3) following hybridization with Secale cereale, the two latter classes of ovules being measured before the primary endosperm nucleus has divided.

TYPE OF OVULE	OVULE NO.	SIZE IN MM³×10,000			
		OVULE	EMBRYO SAC	ANTIPODALS	ANTIPODAL NUCLEI
1. *Hordeum jubatum* (unfertilized)	1	98.52	6.133	2.020	.4752
	2	87.92	6.568	1.695	.3766
	3	101.85	5.754	1.370	.2653
	Av.	96.10	6.152	1.695	.3724
2. *H. jubatum*, selfed	1	189.55	44.595	12.409	1.2498
	2	209.44	43.743	11.219	1.1257
	3	177.07	27.389	6.707	.9289
	4	183.62	48.873	13.205	1.4082
	Av.	189.92	41.150	10.885	1.1781
3. *H. jubatum*×*S. cereale*	1	102.10	9.767	2.752	.4623
	2	132.14	12.833	3.373	.6121
	3	120.97	9.180	2.641	.4579
	4	106.77	9.331	2.230	.4408
	Av.	115.49	10.278	2.749	.4933

from planimeter measurements of outline drawings of the tissues made with the aid of a camera lucida from successive serial sections cut at 15 microns thickness. An accurate estimation of volume is obtained in this way although the procedure is laborious. The measurements are presented in table 1 and illustrated diagrammatically in figure 1. The data are limited in extent but they are very instructive.

Large changes in size of certain tissues within the ovule accompany self-fertilization in *H. jubatum*. A comparison of the values in table 1 for *H. jubatum* selfed with those of the unfertilized ovules shows that total volume of the ovule doubles during the course of gametic union and that of the embryo sac increases over six-fold. The prominent antipodal tissue, occupying about one-quarter of the space in the embryo sac of the unfertilized ovule, likewise increases in size during this interval to over six times its former volume. The

antipodal nuclei enlarge about three-fold. These observations reveal that fertilization of *H. jubatum* following selfing results in a very rapid and pronounced stimulation of the ovule, leading immediately to large increases in size of the embryo sac and antipodals. These changes become manifest before either the primary endosperm nucleus or the zygote divides.

A very different behavior is revealed in the ovules at the same stage when *S. cereale* is used as the staminate parent. The increase at fertilization in size

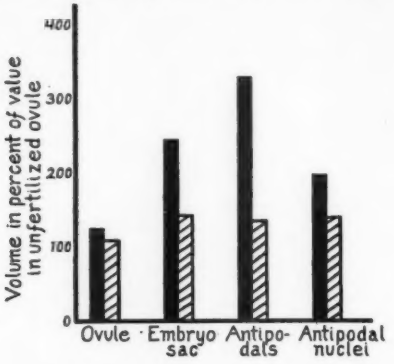

FIG. 2.—Relative volumes at fertilization of the ovule, embryo sac, antipodals and antipodal nuclei following the matings *Hordeum vulgare*, var. Olli, selfed (solid bar), and *H. vulgare* var. Olli×*Secale cereale* (crosshatched bar). Volumes of the four respective tissues in the unfertilized ovule are taken as 100.

of ovule, embryo sac, antipodals and antipodal nuclei is far less than that occurring following self-pollination. The expansion of the embryo sac associated with hybrid fertilization is only about one-eighth that in evidence following selfing and the enlargement of the antipodal tissue is correspondingly small. The antipodal nuclei, which increase in size about three-fold following self-fertilization, are only slightly enlarged following crossing. Evidently rye sperm evokes a much weaker response in the *H. jubatum* ovule than does *H. jubatum* sperm.

THE HORDEUM VULGARE×SECALE CEREALE OVULE

The great enlargement of the antipodals and of the embryo sac in self-fertilized *H. jubatum* normally occurring before the primary endosperm nucleus divides and the failure of rye sperm to stimulate the tissues in like degree appeared to be of basic significance in understanding the cause of failure in the hybrid seeds. The presumed facts were based on such limited data, however, that an independent confirmation of the relation was desirable. Additional material was available for histological study from the mating of common barley, *H. vulgare*, with rye by which the broader validity of the earlier observations could be tested. Data were taken accordingly on volume of ovule, embryo sac, antipodals and antipodal nuclei on five seeds each at the fertiliza-

tion stage following the matings, *H. vulgare* (var. Olli) selfed, and *H. vulgare* var. Olli×*S. cereale*, and on four unfertilized barley ovules of the same variety used in the two matings above. The results are presented in table 2 and figure 2.

It is apparent from the data in table 2 that the relationship observed following the cross *H. jubatum*×*S. cereale* holds also in a similar cross when

TABLE 2

Volume of the Hordeum vulgare ovule and of certain contained structures at (1) the mature embryo sac stage, (2) after self-fertilization and (3) following hybridization with Secale cereale, the two latter classes of ovules being measured before the primary endosperm nucleus has divided.

TYPE OF OVULE	OVULE NO.	SIZE IN MM³×10,000			
		OVULE	EMBRYO SAC	ANTIPODALS	ANTIPODAL NUCLEI
1. *Hordeum vulgare* (unfertilized)	1	286.0	18.221	1.942	.3105
	2	293.6	40.336	5.328	.6832
	3	337.5	45.222	6.284	.7077
	4	368.3	17.759	2.504	.2196
	Av.	321.3	30.384	4.014	.4802
2. *H. vulgare*, selfed	1	360.8	72.951	12.205	.8055
	2	407.4	74.400	13.277	1.0774
	3	457.9	100.148	15.943	1.0705
	4	436.0	74.271	15.127	1.0287
	5	351.9	51.163	9.325	.7636
	Av.	402.8	74.587	13.175	.9491
3. *H. vulgare*×*S. cereale*	1	315.4	28.189	3.703	.6171
	2	250.5	23.965	3.009	.3172
	3	431.3	59.351	7.171	.7392
	4	383.5	54.596	6.319	.8997
	5	376.8	53.672	6.842	.7878
	Av.	351.5	43.955	5.409	.6722

H. vulgare is used as the pistillate parent. With the advent of fertilization there is a striking increase in size of the embryo sac, antipodals and antipodal nuclei in the common barley ovule following selfing and much smaller increases in the corresponding parts of the ovule following crossing with rye.

Mitotic irregularities in the endosperm are a conspicuous feature of development following the *H. vulgare*×*S. cereale* mating; and collapse of this seed occurs earlier than in the other hybrid mating. The responses of the embryo sacs and antipodals to the rye sperm in *H. jubatum* and *H. vulgare* also are similar. Both the immediate results of hybridization and the subsequent gross behavior of the seeds, therefore, are found to be much alike in the two cases.

Hence one may conclude with some confidence that the early effects of crossing described for the few *H. jubatum*×*S. cereale* seeds observed are actually characteristic of the hybrid condition.

SUBSEQUENT BEHAVIOR OF THE ANTIPODALS

The differential stimulation of the *H. jubatum* antipodals at the fertilization stage, depending upon whether the sperm is derived from the same species or

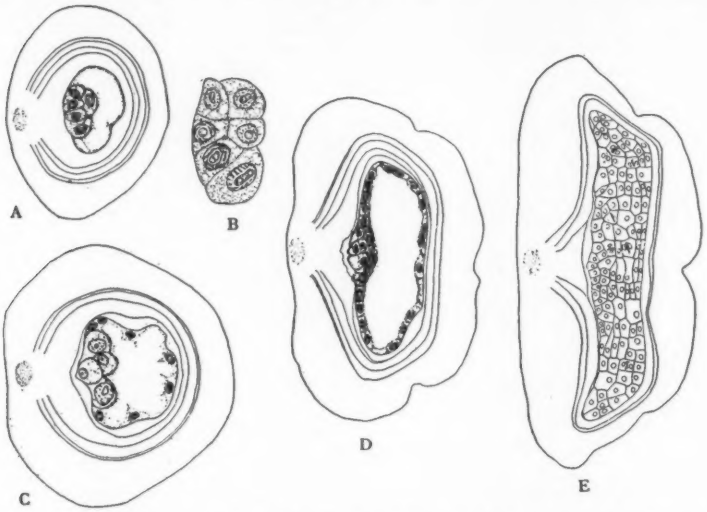

FIG. 3.—Transverse sections of developing grains of *Hordeum jubatum* through the mid-region of the antipodal tissue at stated intervals following self pollination.

A. 10 hours. The antipodal cells and nuclei have greatly increased following fertilization. The cytoplasm has a spongy appearance and vacuoles are forming in the apical portions of the cells. ×69.

B. 10 hours. A group of antipodal cells showing the vacuolate condition of the cytoplasm and the enlarged nuclei. ×142.

C. 28 hours. The antipodal cells are fully extended and the cytoplasm is highly vacuolate. ×69.

D. 48 hours. The antipodal cells are highly vacuolate and shrinking in size. ×69.

E. 72 hours. The antipodal tissue is no longer present. ×69.

from rye, foreshadows striking divergencies in the subsequent behavior of the tissue. The major developmental changes occurring in embryo, endosperm and antipodals of the two classes of seed up to 72 hours after pollination are summarized in table 3, which is based upon the detailed results reported in the preceding paper. We are especially concerned here with the extraordinary contrast in antipodal behavior.

The increase in size of antipodal cells and nuclei in normal *H. jubatum* which, as shown above, is detectable even at the fertilization stage, continues

to about 28 hours. Just prior to fertilization these cells are small and the nuclei are embedded in very dense cytoplasm. Both cells and nuclei have increased greatly in size at ten hours following pollination and many small vacuoles are appearing in the cytoplasm (fig. 3, A and B). At 28 hours this tissue is most

TABLE 3

A summary of development between 10 and 72 hours of normal Hordeum jubatum seeds in contrast with H. jubatum×Secale cereale seeds.

TIME IN HOURS	MATING	EMBRYO	ENDOSPERM	ANTIPODALS
10	*H. jubatum*, selfed	zygote	4- to 8-nucleate	Cells and nuclei much enlarged; prominent vacuoles in cell apices
	H. jubatum×S. cereale	zygote	2- to 4-nucleate	Cells and nuclei variable in size, mostly small; cytoplasm, mostly dense
28	*H. jubatum*, selfed	4 to 6 cells	Typically 64-nucleate	Cells fully extended, highly vacuolate, no increase in number
	H. jubatum×S. cereale	zygote to 4-celled	2 to 16 nuclei varying in sizes; mitosis often aberrant	Cytoplasm mostly dense; some increase in cell size
48	*H. jubatum*, selfed	20 to 30 cells	Cellular near embryo; central cavity enclosed by layer of cells	Shrunken and flattened between endosperm and remnant of nucellus
	H. jubatum×S. cereale	3 to 10 cells	Varying from 2 giant nuclei to 32 or more of variable size	Cells actively dividing; 32 mitotic figures found in one seed
72	*H. jubatum*, selfed	120 cells	Central cavity filled with cells	Have disintegrated, only fragment remaining
	H. jubatum×S. cereale	50 to 80 cells	Very variable in number and size of nuclei; no cell formation	Cells still increasing in number; mitosis is irregular

fully extended (fig. 3 C). There is no increase in number of antipodal cells and nuclei beyond that characteristic of the mature embryo sac stage. After 28 hours the antipodals begin to regress (fig. 3 D); and by 72 hours they are no longer present as an organized structure (fig. 3 E). The antipodals, therefore, are a prominent and presumably active tissue in normal *H. jubatum* throughout the period when the endosperm is free nucleate. They quickly decline when the rapidly developing young endosperm becomes cellular.

The antipodals in the hybrid seeds, on the other hand, change slowly during the period in which their normal counterparts are enlarging rapidly. Furthermore, the early changes in cell and nuclear size are not only small but also uneven and the cytoplasm remains dense with a very few small vacuoles (fig. 4, A and B). The cells are continuing to increase in size at 28 hours. Some of

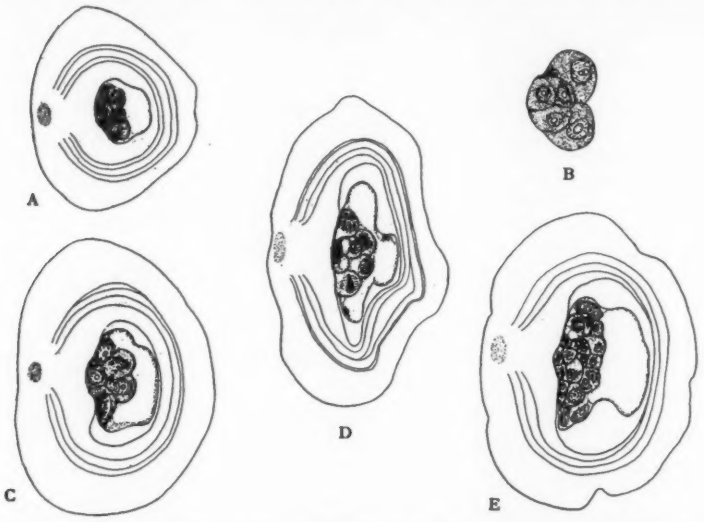

FIG. 4.—Transverse sections of developing grains of *Hordeum jubatum* through the mid-region of the antipodal tissue at like intervals following cross pollination, *Secale cereale* being used as the staminate parent.

A. 10 hours. A few of the antipodal cells increase somewhat in size while the majority remain small. All cells are densely cytoplasmic and there is little evidence of vacuolization. ×69.

B. 10 hours. A group of antipodal cells showing their small and uneven size and the dense nature of their cytoplasm. ×142.

C. 28 hours. The antipodal cells have increased in size and are, for the most part, densely cytoplasmic. Nuclei of two of the cells are in stages of division. ×69.

D. 48 hours. The antipodal tissue is increasing in size due to nuclear and cell division. ×69.

E. 72 hours. A large compact antipodal tissue is formed as a result of the increasingly meristematic condition of its cells. ×69.

the nuclei are in stages of mitosis at this time (fig. 4 C). Nuclear and cell divisions are frequent at 48 hours (fig. 4 D) and continue at 72 hours (fig. 4 E). By 96 hours, however, the hyperplastic antipodal tissue characteristic of the hybrid seed is breaking down.

The metamorphosis of the antipodals in the normal *H. jubatum* seed is further illustrated in figures 5 to 8. These are outline drawings based on 15-micron serial sections of typical seeds and are intended to reflect volume changes. A comparison of figures 5 and 6 shows the marked increase in antipodal size associated with normal fertilization. Figure 7 shows the *H. jubatum* antipodals

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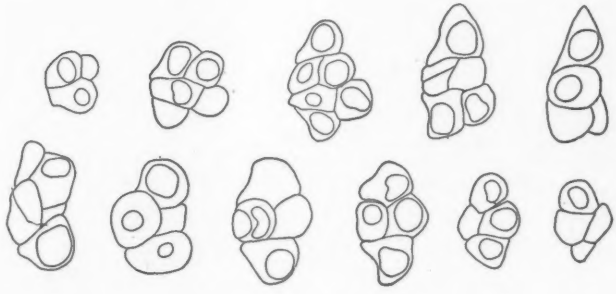

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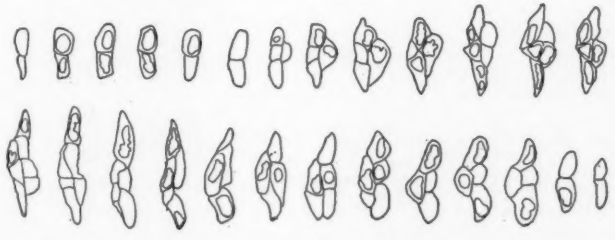

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FIG. 5.—Serial transverse sections of the antipodal tissue showing its size and extent in the mature megagametophyte of *Hordeum jubatum*. ×124.

FIG. 6.—Serial transverse sections of the antipodal tissue from a megagametophyte two hours after self pollination and immediately following fertilization showing the great increase in the size of both cells and nuclei. ×124.

FIG. 7.—A like series at 28 hours following self pollination showing the great increase in size of antipodal tissue. ×124.

FIG. 8.—A like series at 48 hours following self pollination showing the flattened condition of the antipodal cells. ×124.

at their maximum size, 28 hours after pollination. By 48 hours, as illustrated in figure 8, a marked shrinkage of the tissue has taken place.

Only one illustration of the *H. jubatum*×*S. cereale* hybrid is included in this series, namely, figure 9. This is based upon the antipodals in a seed collected at

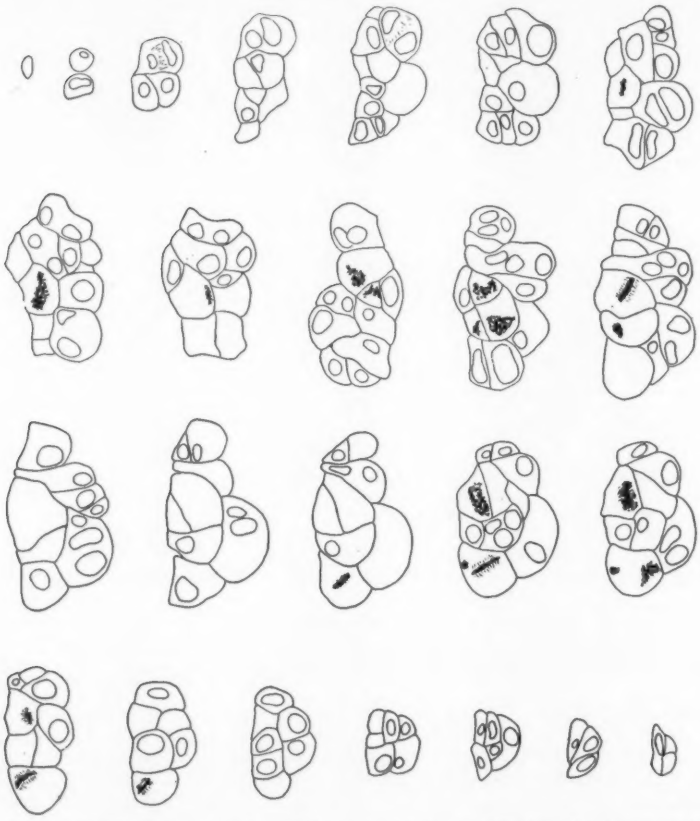
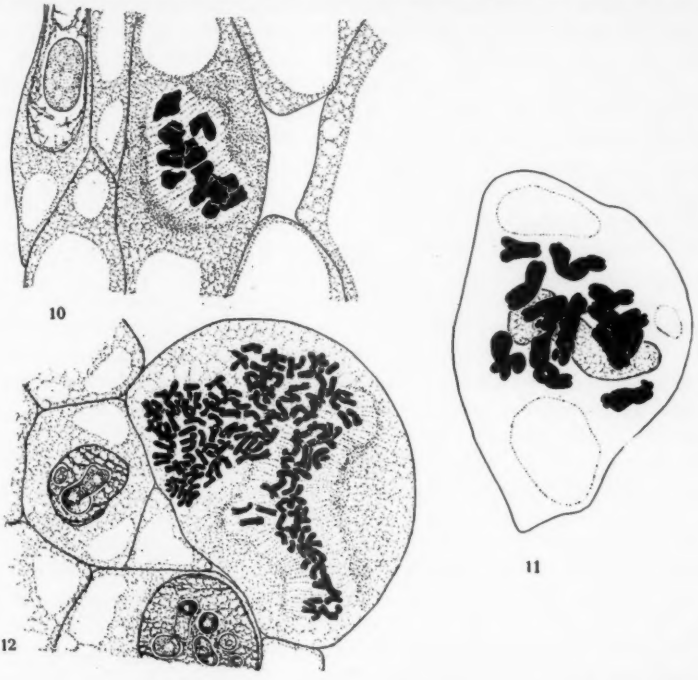


FIG. 9.—Serial transverse sections of the antipodal tissue of *Hordeum jubatum* at 72 hours following pollination using *Secale cereale* as the staminate parent. The tissue is actively meristematic and the cells vary greatly in size and number of nuclei per cell. ×142.

72 hours. The persistence at this comparatively late stage and the large size of the tissue are noteworthy. The cells are irregular in size and several mitotic figures are present. Figure 9 illustrates the maximum development of the antipodals in the hybrid seed.

Two types of division figures are to be found in the antipodals of the hybrid seed, both of which may occur in the same tissue. One type is characterized by

giant chromosomes present in the normal number. This class is represented in figures 10 and 11, showing, respectively, 14 large chromosomes approaching the equatorial plate at 42 hours after pollination and 14 longitudinally split giant chromosomes, 10 hours later, in a cell in which the nuclear membrane has disappeared. The nuclei possessing giant chromosomes do not give rise to



FIGS. 10 and 11.—Mitoses in antipodal cells with 14 giant chromosomes. Figure 10, metaphase at 42 hours after cross pollination; figure 11, late prophase at 52 hours after cross pollination. ×686.

FIG. 12.—Section of a large antipodal cell showing the large number of chromosomes of normal size which may be present in such cells. The metaphase plate was coiled within the cell and extended through six sections, one of which is shown, so that many times the gametophytic number (14) of chromosomes was distributed on it. ×686.

daughter nuclei although division appears to proceed to various stages short of this before being arrested. Considerable hypertrophy of the antipodal nuclei and increase in size of chromosomes occurs normally in *H. jubatum* selfed. This type of antipodal nucleus in the hybrid seed is similar. The most apparent difference, however, is that the nuclei in the normal seed do not show division stages beyond prophase. The second type of antipodal division figure is found in very large cells in which high numbers of chromsomes of normal size are

present. Such an antipodal cell in a hybrid seed taken at 56 hours is shown in figure 12. This cell extended through seven sections cut at 15 microns thickness. The equatorial plate was coiled within the cell and many times the gametophytic number (14) of chromosomes was distributed on it. Figure 12 is based on but one of the six sections in which portions of the equatorial plate were found. This is an extreme case. Numerous other antipodal cells were observed, however, whose nuclei contained greatly increased even if lesser numbers of chromosomes.

The fact is established, therefore, that irregularity in chromosome number in the nuclei of *H. jubatum*×*S. cereale* seeds is not limited to the endosperm, which is of hybrid origin, but may arise also in the antipodal tissue whose nuclear complement is derived entirely from the maternal parent. This circumstance makes it probable that hybridity of the nucleus in itself is not the direct cause of the aberrant mitotic behavior of the endosperm.

DISCUSSION

It has been pointed out in the previous paper that the immediate cause of seed failure following fertilization of *Hordeum jubatum* by *Secale cereale* is starvation arising from impaired development and, eventually, a complete breakdown of the endosperm. The latter tissue appears to be a necessary intermediary between the parent sporophyte and the developing embryo in the nutrition of the latter. Dissolution of the endosperm, however, is not autonomic but takes its origin in profound nuclear disturbances which arise in association with irregular behavior of the antipodals during fertilization and immediately thereafter. Analysis of the problem, therefore, leads back to the relation of the antipodals to development during the crucial period when the mature ovule is undergoing transformation into the juvenile seed. Some of the morphological investigations of an earlier period are pertinent in this connection and since no attempt hitherto has been made to relate them to the general problem of seed failure following species hybridization a brief review will be given.

WESTERMAIER (1890) first showed that the antipodals may play an important role in the nutrition of the embryo. HOFMEISTER (1849), as LLOYD (1902) has noted, had suggested in his classical study on the origin of the embryo in angiosperms that the function of the antipodals was nutritive. It remained for WESTERMAIER, however, to gather the evidence which made such a conclusion apparent. WESTERMAIER directed attention to (1) the position of the antipodals relative to the nutrient stream (2) the location of the cuticularized membranes in the ovule and (3) the distribution of starch in the ovule. Several later investigators have concurred in this general point of view as observations on antipodal behavior have been extended to additional species.

LLOYD (1902) found that development of the antipodals was highly variable, frequently even within families. A detailed study of the Rubiaceae showed that within the Galieae, for example, most of the species possess three antipodals, one of which is much elongated and seems to perform a haustorial function.

The antipodals in the genus Crucianella, on the other hand, are of very short duration and show no special morphological features. Their function, according to LLOYD, is at best but passive and is carried out by the mere giving up of their substance in death to the embryo sac. The antipodals in *Diodia virginiana* vary from four to ten cells which are arranged in a long series physiologically equivalent to the single elongated antipodal in the Galieae. LLOYD observes that in Richardsonia and Haustoria the antipodals take on the appearance of glandular cells and, for a brief period, are active in some kind of secretion.

IKEDA (1902) in a study of the antipodals in *Tricyrtis hirta* observed the extraordinary behavior of the nuclei. As the antipodal cells attain their maximum length at, or just following, fertilization, the nuclei, which are at first small, enlarge greatly and show a very considerable increase in content of chromatin. The latter substance becomes aggregated into dense, highly stainable, usually angular, masses. IKEDA interprets the nuclear behavior as indicative of intensive metabolic activity in the antipodals and suggests that the tissue is the center for the absorption, elaboration and transportation of nutrient materials for the embryo sac. He states that several investigators have observed chromatin aggregation as an accompaniment of secretory activity in glandular cells in both animals and plants. The nutritive function of the antipodals appears to continue to the beginning of endosperm formation at which time the large chromatin masses gradually disappear and the whole tissue regresses. It is probably significant that in Tricyrtis a special group of columnar cells is differentiated in the chalaza connecting the vascular bundle with the embryo sac during the time the antipodals are active and in rather direct physical association with them. These cells doubtless serve for conduction of nutrients and water.

LÖTSCHER (1905) recognizes three general classes of antipodals. Type I shows a low grade of differentiation of the antipodal cells and these probably play only a very minor developmental role. The antipodals form a more prominent tissue in Type II which is effective in the manufacture and transmission of materials required by the embryo sac. This type is represented, among other families, by the Gramineae. The antipodal cells in Type III form an elongated structure, serving the embryo sac as a haustorium. LÖTSCHER considers that the antipodals are a part of the total mechanism by which the embryo is nourished, their share in this task varying considerably between species and standing in reciprocal relation to other structures serving the same end.

The antipodal apparatus in the Gramineae has been studied by SHADOWSKY (1926). The number of cells observed varied from three to 43, species having multiple antipodals preponderating. SHADOWSKY believes that the antipodals function in transmitting nutrients to other parts of the embryo sac and thinks it significant that the tissue persists in this family until the endosperm starts growth.

SCHNARF (1929), in a critical survey, emphasizes the fact that the antipodals are the most variable constituent of the embryo sac in number, form of cell and nucleus and in persistence. Usually three only are present, but additional

ones may be derived from these. The increase in number is often associated with increased size, persistence or hypertrophy of the nuclei. There may be considerable variability in the antipodal apparatus between species within a family. SCHNARF points out that small antipodals usually disappear early whereas large ones may reach their maximum development after fertilization and remain active until the beginning of endosperm formation. He attributes a significant nutritive role to the antipodals in species in which the tissue is well developed. The general grounds for this view are summarized in the following statement from SCHNARF which is quoted in translation:

"(1) The position of the antipodals at the base of the embryo sac where in general the conducting tissue of the ovule terminates points to the assumption that the incoming material must pass through the antipodal region.

"(2) The form and particularly the finer structure of the antipodal cells point to a function similar to that of secretory cells.

"(3) The female gametophyte may be looked upon as an independent individual, so to speak, which is nourished by the plant less directly than a bud or flower; an agency is necessary to take up nutrients from the sporophyte and transform them into other substances which the growing gametophyte may absorb. This agency is frequently the antipodals."

SCHNARF calls attention to the fact that the "hypertrophied" nuclei characterizing highly developed antipodals are not peculiar to this tissue but are found also in anther tapetum, in endosperm, particularly in endosperm haustoria, in the suspensor cells of many embryos and in various other plant parts. This form of nucleus may be indicative of a function common to all these tissues which may well be secretory.

The prominent development of the antipodals athwart the nutrient stream in *Hordeum jubatum*, the rapid enlargement of these cells at fertilization associated with a corresponding increase in size of the embryo sac and the extraordinary changes through which the nuclei and chromosomes pass all point to an active participation of the antipodals in the transformation of the ovule into a young seed. The resemblance of the nuclei of antipodals of this class to those of glandular cells in general, noted by earlier investigators, definitely suggests a metabolic role. Substances necessary for the initial growth of the endosperm are probably built up in the antipodals from materials conveyed thence by the vascular bundle, and then secreted into the endosperm mother cell. The endosperm evidently becomes the dynamic center of the entire structure since growth stops upon its breakdown in the hybrid seed. The antipodals function for a brief period only, but since this period falls at the crucial stage of endosperm initiation their influence may be decisive for the whole subsequent course of seed development.

The sequence and possible character of the events leading to collapse of the *H. jubatum*×*S. cereale* seed may now be considered.

The antipodals are aroused to activity by the advent of normal sperm to the *H. jubatum* embryo sac. The promptness with which the antipodals respond is remarkable. Evidently the fertilization process does not proceed very far

before an effect on the antipodals is produced. Probably the action proceeds from the polar nuclei and their associated sperm. Just prior to fertilization the two polar nuclei are closely adjacent to the egg; but immediately thereafter the three associated nuclei move to a position between the egg and the antipodals, approaching the latter. It is unlikely that the pollen tube, while still within the stylar tissue, is the source of the stimulus because in all embryo sacs which have been found showing enlarged antipodals sperms were visible in conjunction with egg and polar nuclei or fertilization had been completed. However this may be, the fact is that, following entrance of the sperm into the sac, the antipodals normally expand to several times their gametophytic size and the primary endosperm becomes greatly enlarged *before* the fusion nucleus begins to divide. This means that an opportunity is provided for even the first nuclear division in the endosperm to be conditioned by substances proceeding from the activated antipodals.

Now Secale sperm, when substituted at fertilization for Hordeum sperm in the *H. jubatum* ovule, fail to arouse the antipodals to their normal secretory activity. Evidently the stimulating substance which the sperm releases is specific, in some measure, so that the *H. jubatum* antipodals do not react fully to the *S. cereale* product. Hybrid fertilization, however, only slightly delays division of the Hordeum×Secale primary endosperm nucleus. Early development of the hybrid endosperm occurs, therefore, in the presence of partially activated antipodals whose position in the embryo sac is such that they can regulate the nutrient supply. If now it is assumed that the weakly functioning antipodals fail to secrete in sufficient amount some material essential for the growth and division of the endosperm nuclei, an explanation is at hand for the abnormal mitosis which arises in the hybrid tissue at this time. The hybrid seed may live beyond the antipodal stage but recovery of the endosperm is impossible because irregular assortment of the chromosomes is an irreversible change.

The hyperplasia of the antipodals often occurring in the *H. jubatum*×*S. cereale* seeds at 48 to 72 hours after pollination seems best interpreted as a side reaction arising from the irregular distribution of nutrients. Post-fertilization multiplication of antipodal cells appears not to occur when common barley is crossed with rye. Otherwise the course of events leading to seed collapse after the *H. vulgare*×*S. cereale* mating is parallel to that which has been described in detail for *H. jubatum*×*S. cereale*.

SUMMARY

The abnormal chromosome distribution characteristic of the hybrid endosperm in *Hordeum jubatum*×*Secale cereale* seeds is shown to be associated with a radically altered behavior of the antipodals.

The antipodals are prominently developed in the female gametophyte in a position athwart the nutrient stream. Their cells and nuclei are normally stimulated to a marked hypertrophy at fertilization. The antipodals appear to function in the secretion of substances necessary for endosperm growth up

to about 28 hours, after which they rapidly decline. Measurements of the volume show that *Secale cereale* sperm, on entering the *H. jubatum* embryo sac, exert only a feeble stimulus on the antipodals. The cytoplasm remains dense and only a limited and irregular enlargement of the nuclei occurs. The triple fusion hybrid nucleus nevertheless enters upon division promptly and early endosperm development proceeds in association with the weakly functioning antipodals.

It is suggested that the hybrid endosperm nuclei under these conditions fail to receive through the antipodals all the materials necessary for growth and reproduction. The starvation is reflected in gross mitotic disturbances which eventually so impair the endosperm that its development is arrested and the entire seed breaks down.

The fact is established that the time relations between stimulation of the antipodals and division of the primary nucleus (which is frequently abnormal) are such that the latter event could be conditioned by subnormal secretory activity of the antipodal tissue.

Confirmation of this explanation of collapse in *H. jubatum*×*S. cereale* seeds is seen in a similar sequence of changes leading to early seed failure following fertilization of common barley (*H. vulgare*) by rye.

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